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Dated 13 September 2001

Patents Form 1/77

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1/77

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310CT00 E579818-3 D02823
P01/7700 0.00-0026505.8

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

THE PATENT OFFICE
30 OCT 2000
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1. Your reference — RJW/CP5876164

2. Patent application number *(The Patent Office will fill in this part)* — **0026505.8** — 30 OCT 2000

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

KuDOS Pharmaceuticals Limited
327 Cambridge Science Park
Milton Road
Cambridge CB4 4WG

Patents ADP number *(if you know it)*

7624901002

If the applicant is a corporate body, give the country/state of its incorporation — England

4. Title of the invention — Phthalazinone Derivatives

5. Name of your agent *(if you have one)* — MEWBURN ELLIS

Address for service@ in the United Kingdom to which all correspondence should be sent *(including the postcode)*

YORK HOUSE
23 KINGSWAY
LONDON
WC2B 6HP

Patents ADP number *(if you know it)* — 109006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month /year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request?
(Answer "Yes" if:
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	1
Description	79
Claim(s)	
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(Please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Hewlett Ellis Date 30 October 2000

12. Name and daytime telephone number of person to contact in the United Kingdom: Robert Watson 020 7240 4405

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Patents Form 1/77

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CONTINUATION OF 1/77

The Patent Office

Request for grant of a patent

CONTINUATION SHEET

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Maybridge plc
Trevillett
Tintagel
Cornwall PL34 0HW

ADP No: 8012247001

State of incorporation: England

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing *(day/month/year)*

PHTHALAZINONE DERIVATIVES

The present invention relates to phthalazinone derivatives, and their use as pharmaceuticals. In particular, the present invention relates to the use of these compounds to inhibit the activity of the enzyme poly (ADP-ribose)polymerase, also known as poly(ADP-ribose)synthase and poly ADP-ribosyltransferase, and commonly referred to as PARP.

The mammalian enzyme PARP (a 113-kDa multidomain protein) has been implicated in the signalling of DNA damage through its ability to recognize and rapidly bind to DNA single or double strand breaks (D'Amours et al, 1999, Biochem. J. 342: 249-268).

Several observations have led to the conclusion that PARP participates in a variety of DNA-related functions including gene amplification, cell division, differentiation, apoptosis, DNA base excision repair and also effects on telomere length and chromosome stability (d'Adda di Fagagna et al, 1999, Nature Gen., 23(1): 76-80).

Studies on the mechanism by which PARP modulates DNA repair and other processes has identified its importance in the formation of poly (ADP-ribose) chains, within the cellular nucleus (Althaus, F.R. and Richter, C., 1987, ADP-Ribosylation of Proteins: Enzymology and Biological Significance, Springer-Verlag, Berlin). The DNA-bound, activated PARP utilizes NAD to synthesize poly (ADP-ribose) on a variety of nuclear target proteins, including topoisomerase, histones and PARP itself (Rhun et al, 1998, Biochem. Biophys. Res. Commun., 245: 1-10)

Poly (ADP-ribosyl)ation has also been associated with malignant transformation. For example, PARP activity is higher in the isolated nuclei of SV40-transformed fibroblasts, while both leukemic cells and colon cancer cells show higher enzyme activity than the equivalent normal leukocytes and colon mucosa (Miwa et al, 1977, Arch. Biochem. Biophys. 181: 313-321; Burzio et al, 1975, Proc. Soc. Exp. Bioi. Med. 149: 933-938; and Hirai et al, 1983, Cancer Res. 43: 3441-3446).

A number of low-molecular-weight inhibitors of PARP have been used to elucidate the functional role of poly (ADP-ribosyl)ation in DNA repair. In cells treated with alkylating agents, the inhibition of PARP leads to a marked increase in DNA-strand breakage and cell killing (Durkacz et al, 1980, Nature 283: 593-596; Berger, N.A., 1985, Radiation Research, 101: 4-14).

Subsequently, such inhibitors have been shown to enhance the effects of radiation response by suppressing the repair of potentially lethal damage (Ben-Hur et al, 1984, British Journal of Cancer, 49 (Suppl. VI): 34-42; Schlicker et al, 1999, Int. J. Radiat. Bioi., 75: 91-100). PARP inhibitors have been reported to be effective in radio sensitising hypoxic tumour cells (US 5,032,617; US 5,215,738 and US 5,041,653).

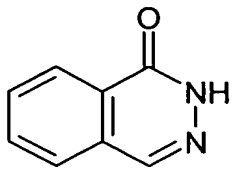
Furthermore, PARP knockout (PARP -/-) animals exhibit genomic instability in response to alkylating agents and γ-irradiation (Wang et al, 1995, Genes Dev., 9: 509-520; Menissier de Murcia et al, 1997, Proc. Natl. Acad. Sci. USA, 94: 7303-7307).

A role for PARP has also been demonstrated in certain vascular diseases, septic shock, ischaemic injury and neurotoxicity (Cantoni et al, 1989, Biochim. Biophys. Acta, 1014: 1-7; Szabo, et al, 1997, J. Clin.Invest., 100: 723-735). Oxygen radical DNA damage that leads to strand breaks in DNA, which are subsequently recognised by PARP, is a major contributing factor to such disease states as shown by PARP inhibitor studies (Cosi et al, 1994, J. Neurosci. Res., 39: 38-46; Said et al, 1996, Proc. Natl. Acad. Sci. U.S.A., 93: 4688-4692). More recently, PARP has been demonstrated to play a role in the pathogenesis of haemorrhagic shock (Liaudet et al, 2000, Proc. Natl. Acad. Sci. U.S.A., 97(3): 10203-10208).

It has also been demonstrated that efficient retroviral infection of mammalian cells is blocked by the inhibition of PARP activity. Such inhibition of recombinant retroviral vector infections was shown to occur in various different cell types (Gaken et al, 1996, J. Virology, 70(6): 3992-4000). Inhibitors of PARP have thus been developed for the use in anti-viral therapies and in cancer treatment (WO91/18591).

Moreover, PARP inhibition has been speculated to delay the onset of aging characteristics in human fibroblasts (Rattan and Clark, 1994, Biochem. Biophys. Res. Comm., 201 (2): 665-672). This may be related to the role that PARP plays in controlling telomere function (d'Adda di Fagagna et al, 1999, Nature Gen., 23(1): 76-80).

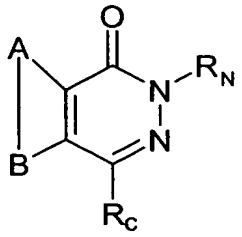
US 5,874,444 discloses a number of PARP inhibitors, amongst which is 1(2H)-phthalazinone (**100**):



(100)

The present inventors have now discovered that derivatives of 1(2H)-phthalazinone and related compounds exhibit stronger inhibition of the activity of PARP than 1(2H)-phthalazinone.

Accordingly, the first aspect of the present invention provides for the use of compounds of the formula:



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, in the preparation of a medicament for inhibiting the activity of PARP, wherein:

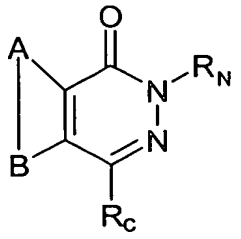
A and B together represent an optionally substituted, fused aromatic ring;

R_C is represented by $-L-R_L$, where L is of formula:

$-(CH_2)_{n1}-Q_{n2}-(CH_2)_{n3}-$

wherein n_1, n_2 and n_3 are each selected from 0, 1, 2 and 3, the sum of n_1, n_2 and n_3 is 1, 2 or 3 and Q is selected from O, S, NH or C(=O); and R_L is selected from optionally substituted C_{3-20} heterocyclyl, C_{5-20} aryl and carbonyl; and R_N is selected from hydrogen, optionally substituted C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl, hydroxy, ether, nitro, amino, thiol, thioether, sulfoxide and sulfone.

The second aspect of the present invention provides for the use of compounds of the formula:



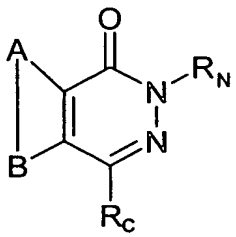
and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, in the preparation of a medicament for inhibiting the activity of PARP, wherein:

A and B together represent an optionally substituted, fused aromatic ring;

R_C is optionally substituted C_{5-20} heteroaryl;

R_N is selected from hydrogen, optionally substituted C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl, hydroxy, ether, nitro, amino, thiol, thioether, sulfoxide and sulfone.

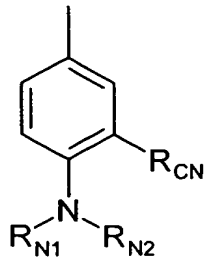
The third aspect of the present invention provides for the use of compounds of the formula:



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, in the preparation of a medicament for inhibiting the activity of PARP, wherein:

A and B together represent an optionally substituted, fused aromatic ring;

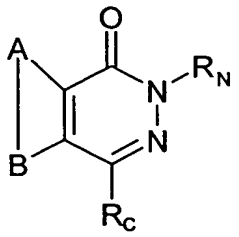
R_C is of the formula



where R_{CN} is selected from amino, nitro and acylamido, and R_{N1} and R_{N2} are independently optionally substituted C_{1-7} alkyl or together form an optionally substituted cyclic amino group; and

R_N is selected from optionally substituted C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl, hydroxy, ether, nitro, amino, thiol, thioether, sulfoxide and sulfone.

The fourth aspect of the present invention provides for the use of compounds of the formula:



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, in the preparation of a medicament for inhibiting the activity of PARP, wherein:

A and B together represent an optionally substituted, fused aromatic ring;

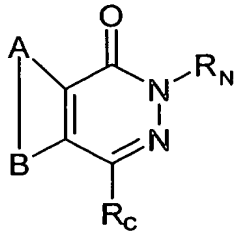
R_C is selected from optionally substituted C_{1-7} alkyl or is represented by $-T-R_T$, where T is of formula:

$-(CH_2)_{t1}-Q_{t2}-(CH_2)_{t3}-$

wherein t_1, t_2 and t_3 are each selected from 0, 1, 2 and 3, the sum of t_1, t_2 and t_3 is 0, 1, 2 or 3 and Q is selected

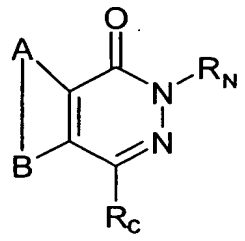
from O, S, NH or C(=O); and R_T is selected from optionally substituted C_{3-20} heterocyclyl, C_{5-20} aryl and carbonyl; and R_N is selected from optionally substituted C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl, hydroxy, ether, nitro, amino, thiol, thioether, sulfoxide and sulfone.

The fifth aspect of the present invention provides for the use of compounds of the formula:



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, in the preparation of a medicament for inhibiting the activity of PARP, wherein:
A and B together represent an optionally substituted, fused aromatic ring, except an unsubstituted benzene ring;
R_C is selected from optionally substituted C_{1-7} alkyl or is represented by $-T-R_T$, where T is of formula:
$-(CH_2)_{t1}-Q_{t2}-(CH_2)_{t3}-$
wherein t_1, t_2 and t_3 are each selected from 0, 1, 2 and 3, the sum of t_1, t_2 and t_3 is 0, 1, 2 or 3 and Q is selected from O, S, NH or C(=O); and R_T is selected from optionally substituted C_{3-20} heterocyclyl, C_{5-20} aryl and carbonyl; and R_N is selected from hydrogen, optionally substituted C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl, hydroxy, ether, nitro, amino, thiol, thioether, sulfoxide and sulfone.

The sixth aspect of the present invention provides for the use of compounds of the formula:



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, in the preparation of a medicament for inhibiting the activity of PARP, wherein:

A and B together represent an optionally substituted, fused aromatic ring;

R_C is phenyl, optionally substituted with 1 or 2 groups independently selected from halo, C_{1-7} alkyl, thiol, thioether, nitro, C_{5-20} heteroaryl, ether, hydroxy and amino; and

R_N is selected from hydrogen, optionally substituted C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl, hydroxy, ether, nitro, amino, thiol, thioether, sulfoxide and sulfone.

Further aspects of the invention provide for the use of compounds as defined in the first to sixth aspects of the invention in the preparation of a medicament for the treatment of: vascular disease; septic shock; ischaemic injury; neurotoxicity; haemorraghic shock; or viral infection.

Another further aspect of the invention provides for the use of compounds as defined in the first to sixth aspects of the invention in the preparation of a medicament for use as an adjunct in cancer therapy.

Definitions

The term "aromatic ring" is used herein in the conventional sense to refer to a cyclic aromatic structure, that is, a cyclic structure having delocalised π-electron orbitals.

The aromatic ring fused to the main core may bear further fused aromatic rings (resulting in, e.g. naphthyl or anthracenyl groups). The aromatic ring(s) may comprise solely carbon atoms, or may comprise carbon atoms and one or more heteroatoms, including but not limited to, nitrogen, oxygen, and sulfur atoms. The aromatic ring(s) preferably have five or six ring atoms.

The aromatic ring(s) may optionally be substituted. If a substituent itself comprises an aryl group, this aryl group is not considered to be a part of the aryl group to which it is attached. For example, the group biphenyl is considered herein to be a phenyl group (an aryl group comprising a single aromatic ring) substituted with a phenyl group. Similarly, the group benzylphenyl is considered to be a phenyl group (an aryl group comprising a single aromatic ring) substituted with a benzyl group.

In one group of preferred embodiments, the aromatic group comprises a single aromatic ring, which has five or six ring atoms, which ring atoms are selected from carbon, nitrogen, oxygen, and sulfur, and which ring is optionally substituted. Examples of these groups include benzene, pyrazine, pyrrole, thiazol, isoxazole, and oxazole. 2-pyrone can also be considered to be an aromatic ring, but is less preferred.

If the aromatic ring has six atoms, then preferably at least four, or even five or all, of the ring atoms are carbon. The other ring atoms are selected from nitrogen, oxygen and sulphur, with nitrogen and oxygen being preferred. Suitable groups include a ring with: no hetero atoms (benzene); one nitrogen ring atom (pyridine); two nitrogen ring atoms (pyrazine, pyrimidine and pyridazine); one oxygen ring atom (pyrone); and one oxygen and one nitrogen ring atoms (oxazine)

If the aromatic ring has five ring atoms, then preferably at least three of the ring atoms are carbon. The remaining ring atoms are selected from nitrogen, oxygen and sulphur. Suitable rings include a ring with: one nitrogen ring atom (pyrrole); two nitrogen ring atoms (imidazole, pyrazole); one oxygen ring atom (furan); one sulphur ring atom (thiophene); one nitrogen and one sulphur ring atom (thiazole); and one nitrogen and one oxygen (isoxazole).

The aromatic ring may bear one or more substituent groups at any available ring position. These substituents are selected from halo, nitro, hydroxy, ether, thiol, thioether, amino, C_{1-7} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl. The aromatic ring may also bear one or more substituent groups which together form a ring. In particular these may be of formula $-(CH_2)_m-$ or $-O-(CH_2)_p-O-$, where m is 2, 3, 4 or 5 and p is 1, 2 or 3.

C_{1-7} alkyl: The term "C_{1-7} alkyl," as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a C_{1-7}hydrocarbon compound having from 1 to 7 carbon atoms, which may be aliphatic or alicyclic, or a combination thereof, and which may be saturated, partially unsaturated, or fully unsaturated.

Examples of (unsubstituted) saturated linear C_{1-7} alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, n-butyl, and n-pentyl (amyl).

Examples of (unsubstituted) saturated branched C_{1-7} alkyl groups include, but are not limited to, iso-propyl, iso-butyl, sec-butyl, tert-butyl, and neo-pentyl.

Examples of saturated alicyclic (carbocyclic) C_{1-7} alkyl groups (also referred to as "C_{3-7} cycloalkyl" groups) include, but are not limited to, unsubstituted groups such as cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl, as well as substituted groups (e.g., groups which comprise such groups), such as methylcyclopropyl, dimethylcyclopropyl, methylcyclobutyl, dimethylcyclobutyl, methylcyclopentyl, dimethylcyclopentyl, methylcyclohexyl, dimethylcyclohexyl, cyclopropylmethyl and cyclohexylmethyl.

Examples of (unsubstituted) unsaturated C_{1-7} alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C_{2-7} alkenyl" groups) include, but are not limited to, ethenyl (vinyl, $-CH=CH_2$), 2-propenyl (allyl, $-CH-CH=CH_2$), isopropenyl ($-C(CH_3)=CH_2$), butenyl, pentenyl, and hexenyl.

Examples of (unsubstituted) unsaturated C_{1-7} alkyl groups which have one or more carbon-carbon triple bonds (also referred to as "C_{2-7} alkynyl" groups) include, but are not limited to, ethynyl (ethinyl) and 2-propynyl (propargyl).

Examples of unsaturated alicyclic (carbocyclic) C_{1-7} alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C_{3-7} cycloalkenyl" groups) include, but

are not limited to, unsubstituted groups such as cyclopropenyl, cyclobutenyl, cyclopentenyl, and cyclohexenyl, as well as substituted groups (e.g., groups which comprise such groups) such as cyclopropenylmethyl and cyclohexenylmethyl.

C_{3-20} heterocyclyl: The term "C_{3-20} heterocyclyl," as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a ring atom of a non-aromatic C_{3-20} heterocyclic compound, said compound having one ring, or two or more rings (e.g., spiro, fused, bridged), and having from 3 to 20 ring atoms, atoms, of which from 1 to 10 are ring heteroatoms, and wherein at least one of said ring(s) is a heterocyclic ring. Preferably, each ring has from 3 to 7 ring atoms, of which from 1 to 4 are ring heteroatoms. "C_{3-20}" denotes ring atoms, whether carbon atoms or heteroatoms.

Examples of C_{3-20} heterocyclyl groups having one nitrogen ring atom include, but are not limited to, those derived from aziridine, azetidine, azetine, pyrrolidine, pyrroline, piperidine, dihydropyridine, tetrahydropyridine, and dihydropyrrole (azoline).

Examples of C_{3-20} heterocyclyl groups having one oxygen ring atom include, but are not limited to, those derived from oxirane, oxetane, oxolane (tetrahydrofuran), oxole (dihydrofuran), oxane (tetrahydropyran), dihydropyran, and pyran. Examples of substituted C_{3-20} heterocyclyl groups include sugars, in cyclic form, for example, furanoses and pyranoses, including, for example, ribose, lyxose, xylose, galactose, sucrose, fructose, and arabinose.

Examples of C_{3-20} heterocyclyl groups having one sulfur ring atom include, but are not limited to, those derived from

thiolane (tetrahydrothiophene, thiane) and tetrahydrothiopyran.

Examples of C_{3-20} heterocyclyl groups having two oxygen ring atoms include, but are not limited to, those derived from dioxane.

Examples of C_{3-20} heterocyclyl groups having two nitrogen ring atoms include, but are not limited to, those derived from diazolidine (pyrazolidine), pyrazoline, imidazolidine, imidazoline, and piperazine.

Examples of C_{3-20} heterocyclyl groups having one nitrogen ring atom and one oxygen ring atom include, but are not limited to, those derived from tetrahydrooxazole, dihydrooxazole, tetrahydroisoxazole, dihydroiosoxazole, morpholine, tetrahydrooxazine, dihydrooxazine, and oxazine.

Examples of C_{3-20} heterocyclyl groups having one oxygen ring atom and one sulfur ring atom include, but are not limited to, those derived from oxathiolane and oxathiane.

Examples of C_{3-20} heterocyclyl groups having one nitrogen ring atom and one sulfur ring atom include, but are not limited to, those derived from thiazoline, thiazolidine, and thiomorpholine.

Other examples of C_{3-20} heterocyclyl groups include, but are not limited to, oxadiazine.

If the C_{3-20} heterocyclyl is substituted, the substituents are on carbon, or nitrogen (if present), atoms.

C_{5-20} aryl: The term "C_{5-20} aryl," as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of a C_{5-20} aromatic compound, said compound having one ring, or two or more rings (e.g., fused), and having from 5 to 20 ring atoms, and wherein at least one of said ring(s) is an aromatic ring. Preferably, each ring has from 5 to 7 ring atoms.

The ring atoms may be all carbon atoms, as in "carboaryl groups," in which case the group may conveniently be referred to as a "C_{5-20} carboaryl" group.

Examples of C_{5-20} aryl groups which do not have ring heteroatoms (i.e., C_{5-20} carboaryl groups) include, but are not limited to, those derived from benzene (i.e., phenyl) (C_6), naphthalene (C_{10}), anthracene (C_{14}), phenanthrene (C_{14}), and pyrene (C_{16}).

Alternatively, the ring atoms may include one or more heteroatoms, including but not limited to oxygen, nitrogen, and sulfur, as in "heteroaryl groups." In this case, the group may conveniently be referred to as a "C_{5-20} heteroaryl" group, wherein "C_{5-20}" denotes ring atoms, whether carbon atoms or heteroatoms. Preferably, each ring has from 5 to 7 ring atoms, of which from 0 to 4 are ring heteroatoms.

Examples of C_{5-20} heteroaryl groups include, but are not limited to, C_5 heteroaryl groups derived from furan (oxole), thiophene (thiole), pyrrole (azole), imidazole (1,3-diazole), pyrazole (1,2-diazole), triazole, oxazole, isoxazole, thiazole, isothiazole, oxadiazole, and oxatriazole; and C_6 heteroaryl groups derived from isoxazine, pyridine (azine), pyridazine (1,2-diazine), pyrimidine (1,3-diazine; e.g., cytosine, thymine, uracil),

pyrazine (1,4-diazine), triazine, tetrazole, and oxadiazole (furazan).

The heteroaryl group may be bonded via a carbon or hetero ring atom.

Examples of C_{5-20} heteroaryl groups which comprise fused rings, include, but are not limited to, C_9 hetercyclic groups derived from benzofuran, isobenzofuran, indole, isoindole; C_{10} heteroaryl groups derived from quinoline, isoquinoline, benzodiazine, pyridopyridine; C_{14} heteroaryl groups derived from acridine and xanthene.

The above C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl groups, whether alone or part of another substituent, may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below

Halo: -F, -Cl, -Br, and -I.

Hydroxy: -OH.

Ether: -OR, wherein R is an ether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7}alkoxy group), a C_{3-20} heterocyclyl group (also referred to as a C_{3-20} hetercyclyloxy group), or a C_{5-20} aryl group (also referred to as a C_{5-20} aryloxy group), preferably a C_{1-7} alkyl group.

Nitro: $-NO_2$.

Cyano (nitrile, carbonitrile): -CN.

Carbonyl: a group of structure -C(=O)-, which includes acyl, carboxy, ester and amido.

Acyl (keto): -C(=O)R, wherein R is an acyl substituent, for example, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylacyl or C_{1-7} alkanoyl), a C_{3-20} heterocyclyl group (also referred to as C_{3-20} heterocyclylacyl), or a C_{5-20} aryl group (also referred to as C_{5-20} arylacyl), preferably a C_{1-7} alkyl group. Examples of acyl groups include, but are not limited to, $-C(=O)CH_3$ (acetyl), $-C(=O)CH_2CH_3$ (propionyl), $-C(=O)C(CH_3)_3$ (butyryl), and -C(=O)Ph (benzoyl, phenone).

Carboxy (carboxylic acid): -COOH.

Ester (carboxylate, carboxylic acid ester, oxycarbonyl): -C(=O)OR, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, $-C(=O)OCH_3$, $-C(=O)OCH_2CH_3$, $-C(=O)OC(CH_3)_3$, and -C(=O)OPh.

Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $-C(=O)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)N(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$, as well as amido groups in which R^1 and R^2, together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.

Amino: $-NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a

C_{1-7} alkyl group, or, in the case of a "cyclic" amino group, R^1 and R^2, taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Examples of amino groups include, but are not limited to, $-NH_2$, $-NHCH_3$, $-NHCH(CH_3)_2$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$, and -NHPh. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, perhydrodiazepino, morpholino, and thiomorpholino. The cylic amino groups may be substituted on their ring by any of the substituents defined here, for example carboxy, carboxylate and amido.

Acylamido (acylamino): $-NR^1C(=O)R^2$, wherein R^1 is an amide substituent, for example, hydrogen, a C_{1-7}alkyl group, a C_{3-20}heterocyclyl group, or a C_{5-20}aryl group, preferably H or a C_{1-7}alkyl group, most preferably H, and R^2 is an acyl substituent, for example, a C_{1-7}alkyl group, a C_{3-20}heterocyclyl group, or a C_{5-20}aryl group, preferably a C_{1-7}alkyl group. Examples of acylamide groups include, but are not limited to, $-NHC(=O)CH_3$, $-NHC(=O)CH_2CH_3$, and -NHC(=O)Ph.

Thiol : -SH.

Thioether (sulfide): -SR, wherein R is a thioether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkylthio group), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of C_{1-7} alkylthio groups include, but are not limited to, $-SCH_3$ and $-SCH_2CH_3$.

Sulfoxide (sulfinyl): -S(=O)R, wherein R is a sulfoxide substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7}

alkyl group. Examples of sulfoxide groups include, but are not limited to, $-S(=O)CH_3$ and $-S(=O)CH_2CH_3$.

Sulfone (sulfonyl): $-S(=O)_2R$, wherein R is a sulfone substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfone groups include, but are not limited to, $-S(=O)_2CH_3$ (methanesulfonyl, mesyl), $-S(=O)_2CF_3$, $-S(=O)_2CH_2CH_3$, and 4-methylphenylsulfonyl (tosyl).

Substituents Form a Ring

It is possible that a substituent on a ring which forms part of R_C and a substituent on the fused aromatic ring (represented by -A-B-), may together form an intra ring link, thus forming a further cyclic structure in the compound.

The substituent on the aromatic ring that forms the intra ring link is preferably on the atom adjacent the central moiety (i.e. at the α-position).

In embodiments of the aspects of the invention where a ring is bound directly to the central moiety, the substituent on R_C that forms the intra ring link is preferably on the atom which is one atom away from the central moiety (i.e. β to the central moiety).

In embodiments of the aspects of the invention where a ring is not bound directly to the central moiety, the substituent on R_C that forms the intra ring link is preferably on the atom which is one atom away from the atom which is bound to the central moiety.

The link between the two rings may be a single bond, or may be of the formula:

$-(CH_2)_{n1'}-Q'_{n2'}-(CH_2)_{n3'}-$

wherein n1', n2' and n3' are each selected from 0, 1, 2 and 3 and the sum of n1', n2' and n3' is less than or equal to 3. Q' can be O, S, NH or C(=O).

It is preferred that R_C is an ring attached directly to the central moiety, and more preferably an aromatic ring, such as benzene.

Further Preferences

In the first to fourth and sixth aspect of the present invention, the fused aromatic ring(s) represented by -A-B- preferably consist of solely carbon ring atoms, and thus may be benzene, naphthalene, and is more preferably benzene. As described above, these rings may be substituted, but are preferably unsubstituted.

In the fifth aspect of the invention, the fused aromatic ring(s) represented by -A-B- preferably consist of solely carbon ring atoms, and thus may be substituted benzene, (optionally substituted) naphthalene, and is more preferably substituted benzene.

In the above aspects of the invention R_N is preferably selected from hydrogen (except in the fourth aspect), and C_{1-7} alkyl, which may be substituted or unsubstituted. In one embodiment, R_N is preferably C_{1-3} alkyl, which may be substituted, for example by a C_{5-20} heterocyclic group. Suitable such groups include cyclic amino groups such as piperidino or morpholino. In another embodiment (except of the fourth aspect), R_N is preferably H.

In the first aspect of the invention, L is preferably of formula:
$-(CH_2)_{n1}-Q_{n2}-$, where n1 is selected from 0, 1, 2 and 3 and n2 is selected from 0 and 1 (where the sum of n1 and n2 is 1, 2 or 3), and more preferably n1 is 1. The most preferred option for L is $-CH_2-$. R_L is preferably C_{5-20} aryl, and more preferably a benzene ring, naphthalene, pyridine or 1,3-benzodioxole. When R_L is a benzene ring, it may be substituted, and preferred substituents include: halo, more preferably fluoro; methoxy; methyl; CF_3; hydroxy; NH_2; and acylamido, where the acyl susbtituent is preferably phenyl. These are usually preferred in the para position.

In the second aspect of the invention, R_C preferably only contains one ring heteroatom which is nitrogen or sulphur. If the ring heteroatom is sulphur it is preferably α to the link to the central moiety of the molecule, and if the ring heteroatom is nitrogen it is preferably β to the link to the central moiety of the molecule. Preferred substituents for these hetero-aromatic moieties include: C_{1-7} alkyl, including methyl and n-butyl; halo, including bromo; tosyl ($-S(=O)_2Ph$); thioether, including SMe and SPh. In the case where the hetero-ring atom is α-sulphur, the ring preferably bears one substituent on the adjacent carbon atom.

In the third aspect of the invention, R_{CN} is preferably amino, more preferably $-NH_2$. R_{N1} and R_{N2} preferably form together a cyclic amino group, e.g. pyrrolidino, piperadino, perhydrodiazepino, morpholino and thiomorpholino. The cyclic amino group may bear further substituents, such as amido, carboxy, ester, but that is less preferred.

In the fourth and fifth aspects of the invention, it is preferred that R_C is as defined in any of the first, second, third and sixth aspects of the invention, or more preferably in the preferences expressed here.

In the sixth aspect of the invention, the optional phenyl substituents are preferably selected from halo, C_{1-7} alkyl (more preferably methyl or ethyl), thiol, thioether, nitro, C_{5-20} heteroaryl and ether.

Where appropriate, the above preferences may be taken in combination with each other.

Includes Other Forms

Included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid (-COOH) also includes the anionic (carboxylate) form ($-COO^-$), a salt or solvate thereof, as well as conventional protected forms. Similarly, a reference to an amino group includes the protonated form ($-N^+HR^1R^2$), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic form ($-O^-$), a salt or solvate thereof, as well as conventional protected forms of a hydroxyl group.

Isomers, Salts, Solvates, Protected Forms, and Prodrugs

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diasteriomeric, epimeric, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+)

and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers," as used herein, are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, $-OCH_3$, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, $-CH_2OH$. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g., C_{1-7}alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol, imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hyroxyazo, and nitro/aci-nitro.

Particularly relevant to the present invention is the tautomeric pair that exists when R_N is H, illustrated below:

Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^{1}H, ^{2}H (D), and ^{3}H (T); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g., asymmetric synthesis) and separation (e.g., fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

Unless otherwise specified, a reference to a particular compound also includes ionic, salt, solvate, and protected forms of thereof, for example, as discussed below.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge et al., 1977, "Pharmaceutically Acceptable Salts," J. Pharm. Sci., Vol. 66, pp. 1-19.

For example, if the compound is anionic, or has a functional group which may be anionic (e.g., -COOH may be $-COO^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na+ and K+, alkaline earth cations such as Ca^{2+} and Mg^{2+}, and other cations such as Al^{+3}. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH_4^+) and substituted ammonium ions (e.g., NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

If the compound is cationic, or has a functional group which may be cationic (e.g., $-NH_2$ may be $-NH_3^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous. Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: acetic, propionic, succinic, gycolic, stearic, palmitic, lactic, malic, pamoic, tartaric, citric, gluconic, ascorbic, maleic, hydroxymaleic, phenylacetic, glutamic, aspartic, benzoic, cinnamic, pyruvic, salicyclic, sulfanilic, 2-acetyoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethanesulfonic, ethane disulfonic, oxalic, isethionic, valeric, and gluconic. Examples of suitable polymeric anions include, but are not

limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g., active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form. The term "chemically protected form," as used herein, pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions, that is, are in the form of a protected or protecting group (also known as a masked or masking group or a blocked or blocking group). By protecting a reactive functional group, reactions involving other unprotected reactive functional groups can be performed, without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, Protective Groups in Organic Synthesis (T. Green and P. Wuts, Wiley, 1991).

For example, a hydroxy group may be protected as an ether (-OR) or an ester (-OC(=O)R), for example, as: a t-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (-OC(=O)CH_3, -OAc).

For example, an aldehyde or ketone group may be protected as an acetal or ketal, respectively, in which the carbonyl group (>C=O) is converted to a diether (>$C(OR)_2$), by reaction with, for example, a primary alcohol. The aldehyde or ketone group is readily regenerated by hydrolysis using a large excess of water in the presence of acid.

For example, an amine group may be protected, for example, as an amide or a urethane, for example, as: a methyl amide (-NHCO-CH_3); a benzyloxy amide (-NHCO-$OCH_2C_6H_5$, -NH-Cbz); as a t-butoxy amide (-NHCO-$OC(CH_3)_3$, -NH-Boc); a 2-biphenyl-2-propoxy amide (-NHCO-$OC(CH_3)_2C_6H_4C_6H_5$, -NH-Bpoc), as a 9-fluorenylmethoxy amide (-NH-Fmoc), as a 6-nitroveratryloxy amide (-NH-Nvoc), as a 2-trimethylsilylethyloxy amide (-NH-Teoc), as a 2,2,2-trichloroethyloxy amide (-NH-Troc), as an allyloxy amide (-NH-Alloc), as a 2(-phenylsulphonyl)ethyloxy amide (-NH-Psec); or, in suitable cases, as an N-oxide (>NO•).

For example, a carboxylic acid group may be protected as an ester for example, as: an C_{1-7}alkyl ester (e.g., a methyl ester; a t-butyl ester); a C_{1-7}haloalkyl ester (e.g., a C_{1-7}trihaloalkyl ester); a triC_{1-7}alkylsilyl-C_{1-7}alkyl ester; or a C_{5-20}aryl-C_{1-7}alkyl ester (e.g., a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

For example, a thiol group may be protected as a thioether (-SR), for example, as: a benzyl thioether; an acetamidomethyl ether (-S-$CH_2NHC(=O)CH_3$).

It may be convenient or desirable to prepare, purify, and/or handle the active compound in the form of a prodrug. The term "prodrug," as used herein, pertains to a compound

which, when metabolised (e.g., in vivo), yields the desired active compound. Typically, the prodrug is inactive, or less active than the active compound, but may provide advantageous handling, administration, or metabolic properties.

For example, some prodrugs are esters of the active compound (e.g., a physiologically acceptable metabolically labile ester). During metabolism, the ester group (-C(=O)OR) is cleaved to yield the active drug. Such esters may be formed by esterification, for example, of any of the carboxylic acid groups (-C(=O)OH) in the parent compound, with, where appropriate, prior protection of any other reactive groups present in the parent compound, followed by deprotection if required. Examples of such metabolically labile esters include those wherein R is C_{1-7}alkyl (e.g., -Me, -Et); C_{1-7}aminoalkyl (e.g., aminoethyl; 2-(N,N-diethylamino)ethyl; 2-(4-morpholino)ethyl); and acyloxy-C_{1-7}alkyl (e.g., acyloxymethyl; acyloxyethyl; e.g., pivaloyloxymethyl; acetoxymethyl; 1-acetoxyethyl; 1-(1-methoxy-1-methyl)ethyl-carbonxyloxyethyl; 1-(benzoyloxy)ethyl; isopropoxy-carbonyloxymethyl; 1-isopropoxy-carbonyloxyethyl; cyclohexyl-carbonyloxymethyl; 1-cyclohexyl-carbonyloxyethyl; cyclohexyloxy-carbonyloxymethyl; 1-cyclohexyloxy-carbonyloxyethyl; (4-tetrahydropyranyloxy) carbonyloxymethyl; 1-(4-tetrahydropyranyloxy)carbonyloxyethyl; (4-tetrahydropyranyl)carbonyloxymethyl; and 1-(4-tetrahydropyranyl)carbonyloxyethyl). Further suitable prodrug forms include phosphonate and glycolate salts.

Also, some prodrugs are activated enzymatically to yield the active compound, or a compound which, upon further chemical reaction, yields the active compound. For example, the

prodrug may be a sugar derivative or other glycoside conjugate, or may be an amino acid ester derivative.

Compounds and their use as pharmaceuticals

Further aspects of the present invention relate to compounds as described in the first to sixth aspects of the invention above, along with, or alternatively, their use as pharmaceuticals.

In general, these aspects relate any of the groups of compounds identified as being preferred in the first to sixth aspects of the present invention.

Particular groups of compounds to which these further aspects relate include:

(i) compounds of the first aspect of the invention where R_L is C_{5-20} aryl, wherein the aromatic ring is substituted by fluorine;

(ii) compounds of the second aspect of the invention where R_C is a five membered ring with a single ring nitrogen β to the link to the central moiety of the molecule; and

(iii) compounds of the third aspect of the invention, except where R_{CN} is $-NO_2$, R_{N1} and R_{N2} together form a piperidino with no substituents.

Acronyms

For convenience, many chemical moieties are represented using well known abbreviations, including but not limited to, methyl (Me), ethyl (Et), n-propyl (nPr), iso-propyl (iPr), n-butyl (nBu), tert-butyl (tBu), n-hexyl (nHex), cyclohexyl (cHex), phenyl (Ph), biphenyl (biPh), benzyl (Bn), naphthyl (naph), methoxy (MeO), ethoxy (EtO), benzoyl (Bz), and acetyl (Ac).

For convenience, many chemical compounds are represented using well known abbreviations, including but not limited to, methanol (MeOH), ethanol (EtOH), iso-propanol (i-PrOH), methyl ethyl ketone (MEK), ether or diethyl ether (Et_2O), acetic acid (AcOH), dichloromethane (methylene chloride, DCM), trifluoroacetic acid (TFA), dimethylformamide (DMF), tetrahydrofuran (THF), and dimethylsulfoxide (DMSO).

Synthesis

Compounds as described in the first to sixth aspects can be synthesised by a number of methods, examples of which are given below. Some synthesis routes are shown in Yamaguchi, et al., *J. Med. Chem.* **1993**, *36*, 4502 - 4068, which is herein incorporated by reference.

In general, a key step in the synthesis of these compounds is the addition/insertion of hydrazine, thus providing the adjacent nitrogen ring atoms in the central moiety. This addition of hydrazine can be accomplished in a ring cyclisation step, as in route 1, part 3 or by a ring insertion step as in route 2, part 2.

As an alternative, the synthesis of these compounds may start with the structure of the core moiety already in place, as in route 3, where a compound such as 1,4-dichlorophthalazine is used as the starting material.

The formed aromatic ring (represented by -A-B-) is usually derivaised before the routes shown below, and starting materials with the desired structure and substituent pattern are either commercially available or readily synthesised.

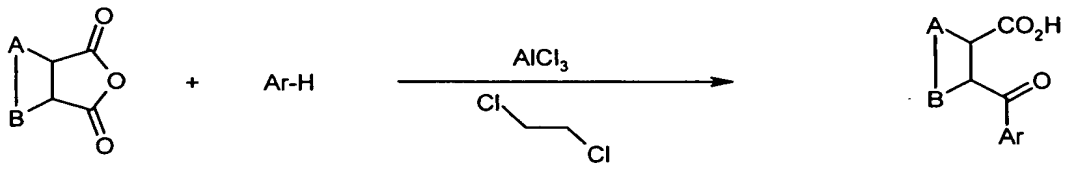
The three main routes illustrated lead to compounds where R_N is H. The possible substituents at this position can be added by the use of an appropriate electrophile with suitable reaction conditions. Some examples of these are given in 'Further derivitisation steps', part (e).

Further derivatisation of the groups on R_C can be carried out using conventional methods, some of which are illustrated in 'Further derivitisation steps'.

Route 1

Part 1: Synthesis of aroylbenzoic acids.

Method A:

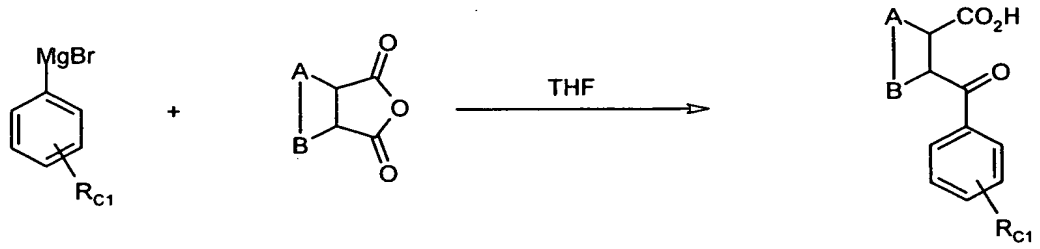


Ar = C_{5-20} aryl

To a stirred solution of aluminium chloride (14.66 g; 0.11 mol) in dichloroethane (150 ml) was added phthalic anhydride (when -A-B- is a benzene ring) (14.81 g; 0.1 mol) in portions. The solution was then stirred at room temperature while a solution of the aromatic electrophile (0.1 mol) in the minimum dichloroethane was added dropwise with stirring with the temperature kept below 30°C. The mixture is then stirred at room temperature for the required time and monitored by T.L.C. After complete reaction the mixture was poured onto ice:water:HCl (250 g: 100 ml: 50 ml) and stirred for 30 mins. The solid was then filtered off through Celite (TRADE MARK) and washed with chloroform (200

ml). The organic layer was removed and the aqueous layer extracted with chloroform (75 ml). The combined organic layers were then extracted with sodium hydroxide (10%; 3 x 100 ml) and the aqueous layers combined. The aqueous solution was then acidified with conc. HCl and extracted with chloroform (3 x 100 ml). The combined organic layers were then dried ($MgSO_4$), filtered and evaporated. The crude solid was then recrystallised from the appropriate solvent or used crude.

Method B:



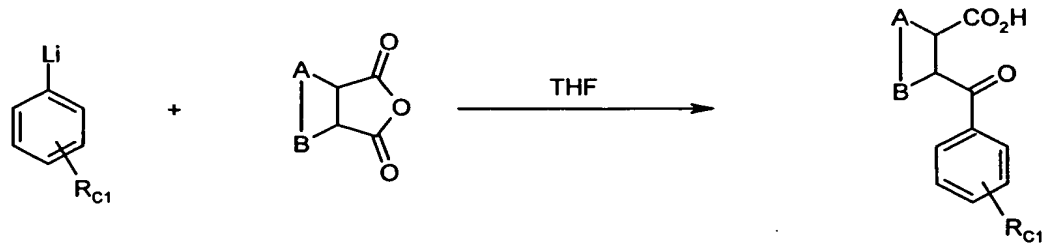
R_{C1} = halo

To a stirred suspension of magnesium (2.67 g; 0.11 mol) in dry THF (150 ml) under nitrogen was added dropwise a solution of the haloaromatic (0.1 mol) in THF (25 ml) until the reaction initialised. The addition rate is then adjusted to maintain a steady reflux. After the addition was completed the solution was cooled to room temperature and a solution of phthalic anhydride (when -A-B- is a benzene ring) (14.81 g; 0.1 mol) in THF (25 ml) is added dropwise with the temperature kept below 20°C during the addition. After complete addition the reaction is stirred at room temperature for 3 h, and then poured onto water/HCl (300 ml water and 20 ml conc. HCl). The THF is then evaporated off and the aqueous layer extracted with

chloroform (3 x 100 ml). The combined organic layers were then extracted with sodium hydroxide (10%; 3 x 100 ml) and the aqueous layer combined. The aqueous solution was then acidified with conc. HCl and extracted with chloroform (3 x 100 ml). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. The crude solid was then recrystallised from the appropriate solvent or used crude.

If -A-B- represents pyrazine, then a slightly different method can be used, without the need for a haloaromatic. To a stirred solution of pyrazine-2,3-dicarboxylic anhydride (10.00 g; 67.0 mmol) in dry THF (84 ml) under nitrogen was added a solution of phenyl magnesium bromide in THF (2M; 67.0 mmol) with the temperature kept below 15°C. The reaction was then stirred at room temperature for 30 min before pouring into water (250 ml) and adding potassium carbonate (10 g). The mixture was extracted with ethyl acetate (3 x 100 ml) and the aqueous layer acidified with HCl (10%) and extracted with ethyl acetate (3 x 100 ml). The extracts were combined, washed with water (100 ml), dried ($MgSO_4$), and evaporated.

Method C:

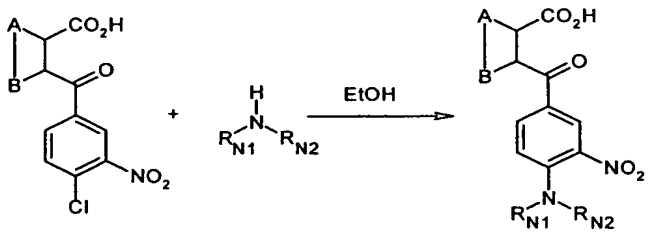


R_{C1} = halo

To a stirred solution of the haloaromatic (0.1 mol) in THF (200 ml) at -78°C under nitrogen was added *n*-butyl lithium

(0.1 mol; 40 ml of a 2.5M solution in hexanes) with the temperature kept below -60°C. The mixture was stirred at 8°C for 1 h before a solution of phthalic anhydride (when -A-B- is a benzene ring) (14.81 g; 0.1 mol) in THF 25 ml) was added with the temperature kept below -60°C. The solution was then stirred at -78°C for 15 min before warming to room temperature and stirring for a further 2h. The mixture was then poured onto water/HCl (300 ml water and 20 ml conc. HCl). The THF is then evaporated off and the aqueous layer extracted with chloroform (3 x 100 ml). The combined organic layers were then extracted with sodium hydroxide (10%; 3 x 100 ml) and the aqueous layer combined. The aqueous solution was then acidified with conc. HCl and extracted with chloroform (3 x 100 ml). The combined organic layers were then dried ($MgSO_4$), filtered and evaporated. The crude solid was then recrystallised from the appropriate solvent or used crude.

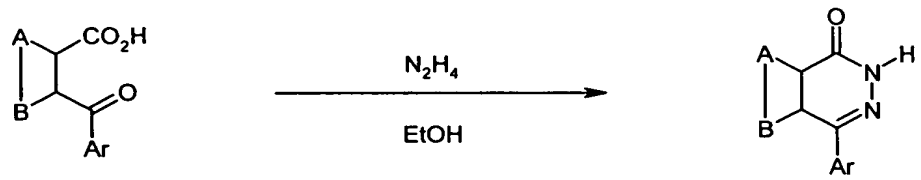
Part 2: Derivatisation of aroylbenzoic acids



A stirred solution of 2-(4-chloro-3-nitrobenzoyl)benzoic acid (45.85 g ;0.15 mol) and the appropriate amine (0.45 mol) in ethanol (300 ml) was heated to reflux for 4 h before cooling to room temperature and pouring onto ice/water (500 g ice and 200 ml water). The aqueous mixture was then stirred rapidly and the mixture acidified with conc. HCl and stirred for a further 30 min. The mixture was allowed to stand and the aqueous layer decanted

off. The oil was then washed with water (200 ml) and then dissolved in ethanol (400 ml).

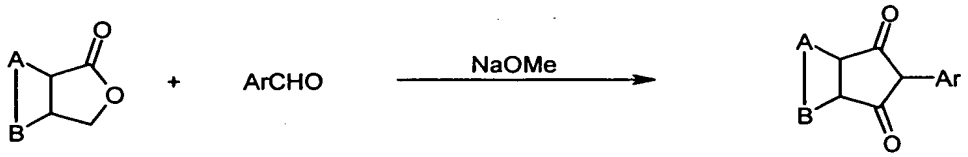
Part 3: Cyclisation of Aroylbenzoic Acids.



A stirred solution of hydrazine monohydrate (5.01 g; 0.1 mol) and the aroylbenzoic acid (0.1 mol) in ethanol (350 ml) was heated to reflux for the required time and monitored by T.L.C. After complete reaction the mixture was cooled and the product filtered off. The solid was recrystallised from ethanol/DMF.

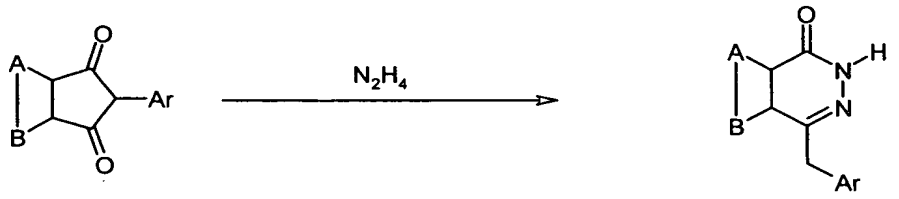
Route 2

Part 1: Synthesis of 2-arylindan-1,3-diones



To an ice-cooled solution of phthalide or equivalent, (13.41 g; 0.1 mol) and the aromatic aldehyde (0.1 mol) in a mixture of methanol (50 ml) and ethylpropionate (50 ml) was added a solution of sodium methoxide in methanol [sodium (9.20 g; 0.4 mol) in methanol (50 ml)] with the temperature kept below 10°C. The solution was then heated to gentle reflux for 3 h, cooled to room temperature and poured onto water (500 ml). The mixture was extracted with ether (5 x 100 ml) and the aqueous layer acidified with acetic acid and the solid filtered off. This was then used crude in the next stage.

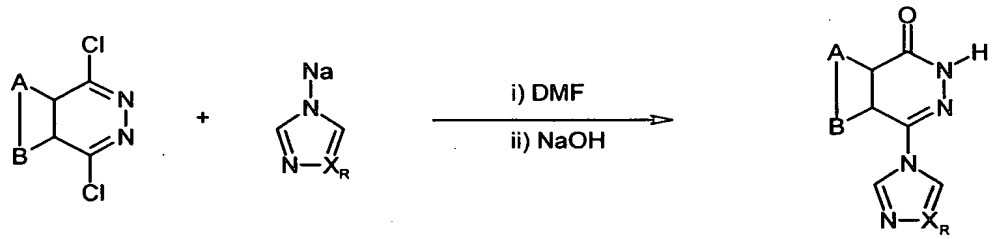
Part 2: Reaction of 2-arylindan-1,3-ones with hydrazine hydrate



A suspension of 2-arylindan-1,3-dione (20 mmol) in hydrazine monohydrate (40 ml) was heated to reflux for 4 h, cooled and the product filtered off. The solid was washed with ethanol.

Route 3

Reaction of sodioimidazole or sodiotriazole with 1,4-dichlorophthalazine (or equivalent)



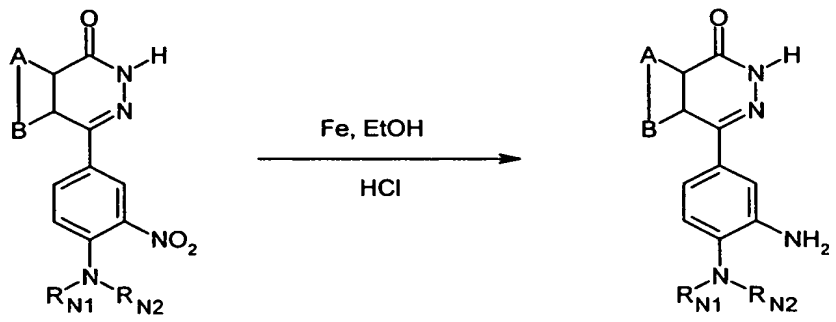
X_R = N or CH

To a stirred solution of imidazole (or 1,2,4-triazole) (10.14 mmol) in dry DMF(20 ml) was added sodium hydride (60% in mineral oil; 0.48 g; 12.06 mmol). The mixture was allowed to stir at room temperature for 1 h before being added dropwise to a stirred solution of 1,4-dichlorophthalazine (2.00 g; 10.05 mmol) in dry DMF (75 ml) with the temperature kept below 10°C. The reaction mixture was stirred at room temperature for 2 h before sodium hydroxide (1.00 g in 20 ml water) was added and the mixture allowed to stir at room

temperature for a further 2 h. The mixture was poured onto dilute hydrochloric acid (50 ml water and 30 ml conc. HCl) and extracted with ethyl acetate (2 x 100 ml). The aqueous layer was then adjusted to pH 8 with solid sodium carbonate and the precipitate filtered off and recrystallised from ethanol.

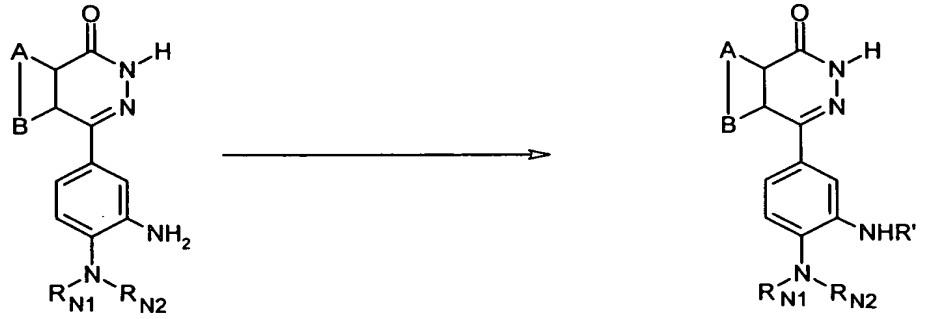
Further derivatisation steps

(a) *Reduction of nitro- group in (4-tert-amino-3-nitrophenyl)phthalazinones*



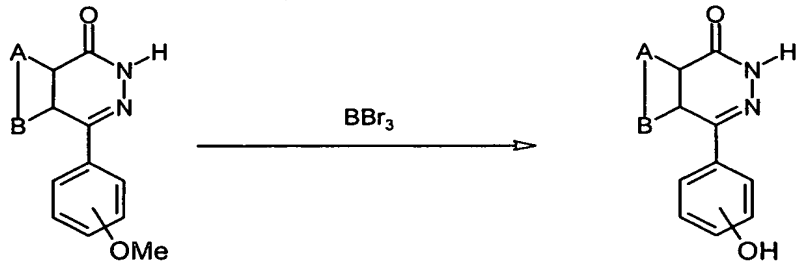
A stirred suspension of the nitro-compound (5.70 mmol) in dilute hydrochloric acid (10%; 100 ml) was heated to approx. 90°C and reduced iron powder (1.59 g; 28.50 mmol) added in portions. After complete addition the mixture was heated at 90°C for 2 h before cooling to room temperature and decanting the suspension of the product away from the iron powder. The solid was filtered off and stirred with sodium hydroxide (10%; 100 ml) for 1 h before the solid was filtered off, washed with water (50 ml) and recrystallised from ethanol/DMF.

(b) *Derivatisation of the amino group in (4-tert-amino-3-aminophenyl)phthalazinones*



To a stirred solution of the (4-*tert*-amino-3-aminophenyl)phthalazinone or equivalent (2.4 mmol) and triethylamine (0.24 g; 2.4 mmol) in 1,4-dioxan (50 ml) was added dropwise the appropriate electrophile [either an acid chloride or a sulfonyl chloride] (2.4 mmol). The mixture was then heated to reflux for 2 h, cooled and poured onto water (100 ml). The solid was then filtered of and washed with water and ethanol before drying *in vacuuo*.

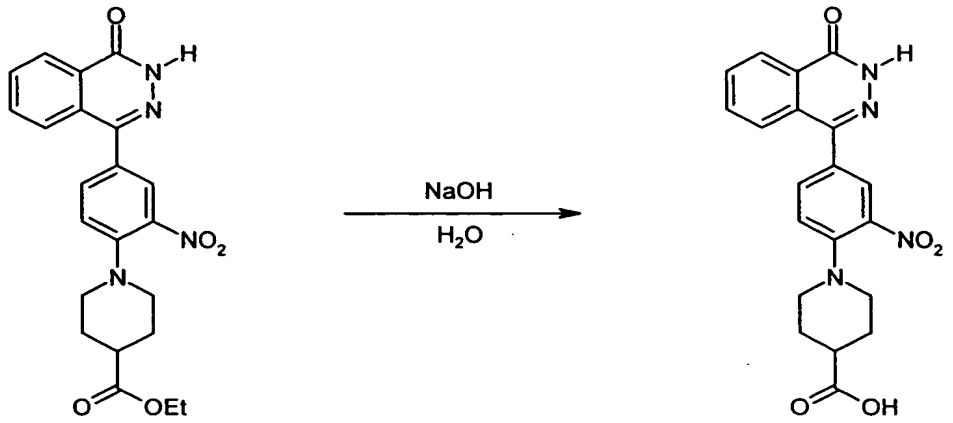
(c) *Demethylation of 1 (2H)-4-(methoxyphenyl)phthalazinone*



A stirred suspension of the appropriate starting compound (e.g. **104**) (2.52 g; 10 mmol) in a solution of boron tribromide (23 ml of a 1M solution in dichloromethane; 23 mmol) under nitrogen was heated to reflux for 24 h, cooled to room temperature, and poured into sodium hydroxide (10%; 100 ml). The organic layer was removed and the aqueous

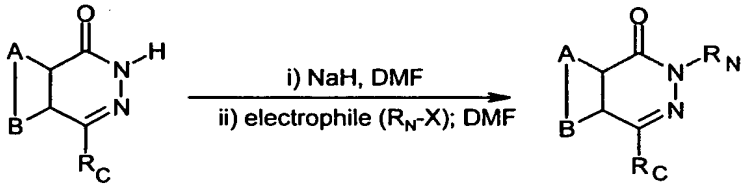
layer washed with dichloromethane (50 ml). The aqueous layer was acidified (10% HCl) and the solid filtered off and washed with water then ethanol.

(d) *Saponification of* ***110***



A stirred suspension of 110 (1.50 g; 3.6 mmol) in sodium hydroxide solution (0.16 g sodium hydroxide in water 30 ml) was heated to 50°C with stirring for 1 h before cooling to room temperature and acidifying the solution with HCl. The product was then filtered off and washed with water.

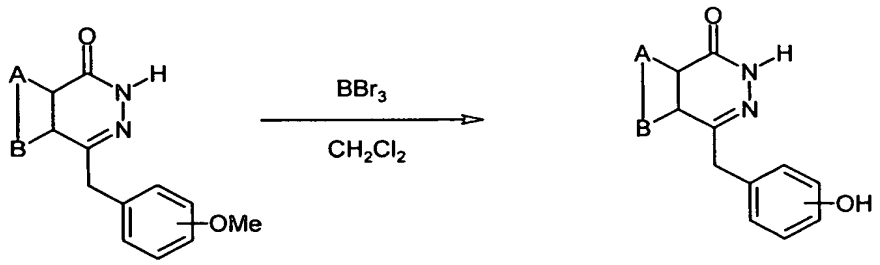
(e) D*erivatisation of the amide group of 1-(2H)phthalazinones*



To a stirred suspension of sodium hydride (1.1 equiv.) in dry DMF (0.5M solution w.r.t NaH) was added the 1-(2*H*)phthalazinone or equivalent (1.0 equiv.). The solution was then stirred at room temperature for 1 h before a solution of the electrophile (1.0 equiv.) in the minimum dry DMF was added. The suspension was then stirred at room

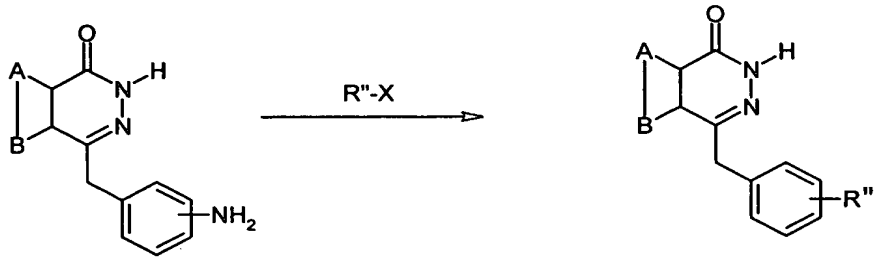
temperature for 3 h, poured into water and the product filtered off. The solid was then washed with water and ethanol before drying *in vacuuo*.

(f) *Demethylation of 4-(methoxybenzyl)- 1(2H)-phthalazinones*



To a suspension of the methoxybenzylphthalazinone or equivalent (0.7 g; 2.65 mmol) in dichloromethane (5 ml) under nitrogen at room temperature was added a solution of boron tribromide in dichloromethane (1M; 6 ml; 6.0 mmol). The mixture was heated to reflux for 24 h, cooled and poured into sodium hydroxide (10%; 25 ml). The organic layer was removed and the aqueous layer acidified (HCl) and the solid filtered off.

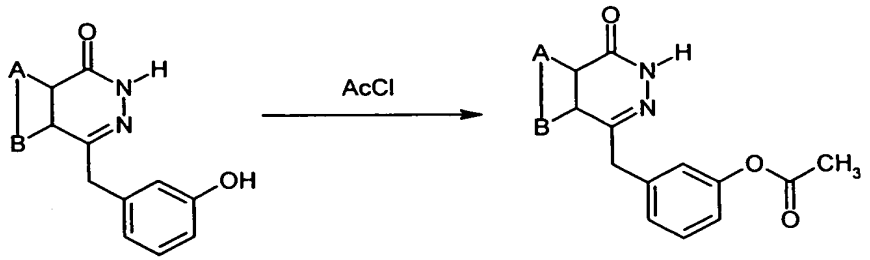
(g) *Derivatisation of 4-(aminobenzyl)- 1(2H)-phthalazinones*



To a stirred solution of the aminobenzylphthalazinone or equivalent (0.6 g; 2.4 mmol) and triethylamine (0.24 g; 2.4 mmol) in 1,4-dioxan (50 ml) was added dropwise the electrophile (2.4 mmol). The mixture was then heated to reflux for 2 h, cooled and poured onto water (100 ml). The solid was then filtered of and washed with water and ethanol

before drying *in vacuuo*.

(h) *Synthesis of 4-(3-O-acetylbenzyl)-1(2H)-phthalazinone*



To a stirred solution of the hydroxybenzylphthalazinone or equivalent (e.g. **164**) (0.7 g; 2.79 mmol) and triethylamine (0.28 g; 2.79 mmol) in 1,4-dioxan (40 ml) was added dropwise acetyl chloride (0.2 ml; 2.79 mmol). The mixture was then heated to reflux for 2 h, cooled and poured onto water (100 ml). The solid was then filtered of and washed with water and ethanol before drying *in vacuuo*.

(i) *Hydrolysis of **143***

To a stirred solution of **143** (0.22 g; 0.63 mmol) in 1,4-dioxan (15 ml) was added sodium hydroxide (5 M; 3 ml). The mixture was stirred at room temperature for 48 h before removing the aqueous layer and extracting it with ethyl acetate (3 x 25 ml). The organic extracts were combined and washed with water (25 ml) before drying ($MgSO_4$), and evaporating.

Use

The present invention provides active compounds, specifically, active in inhibiting the activity of PARP.

The term "active," as used herein, pertains to compounds which are capable of inhibiting PARP activity, and

specifically includes both compounds with intrinsic activity (drugs) as well as prodrugs of such compounds, which prodrugs may themselves exhibit little or no intrinsic activity.

One assay which may conveniently be used in order to assess the PARP inhibition offered by a particular compound is described in the examples below.

The present invention further provides a method of inhibiting the activity of PARP in a cell, comprising contacting said cell with an effective amount of an active compound, preferably in the form of a pharmaceutically acceptable composition. Such a method may be practised *in vitro* or *in vivo*.

For example, a sample of cells may be grown *in vitro* and an active compound brought into contact with said cells, and the effect of the compound on those cells observed. As examples of "effect," the amount of DNA repair effected in a certain time may be determined. Where the active compound is found to exert an influence on the cells, this may be used as a prognostic or diagnostic marker of the efficacy of the compound in methods of treating a patient carrying cells of the same cellular type.

Additional Uses

The invention further provides active compounds for use in a method of treatment of the human or animal body. Such a method may comprise administering to such a subject a therapeutically-effective amount of an active compound, preferably in the form of a pharmaceutical composition. Such use may comprise using the active compounds as

adjuncts.

The term "treatment," as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g., in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e., prophylaxis) is also included.

The term "adjunct" as used herein relates to the use of active compounds in conjunction with known therapeutic means. Such means include cytotoxic regimes of drugs and/or ionising radiation as used in the treatment of different cancer types.

The term "therapeutically-effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio.

The invention further provides a method of treatment of the human or animal body, the method comprising administering to a subject in need of treatment a therapeutically-effective amount of an active compound, preferably in the form of a pharmaceutical composition.

Active compounds may also be used as cell culture additives to inhibit PARP, for example, in order to radio-sensitize

cells to known chemo or ionising radiation treatments in vitro.

Active compounds may also be used as part of an in vitro assay, for example, in order to determine whether a candidate host is likely to benefit from treatment with the compound in question.

Administration

The active compound or pharmaceutical composition comprising the active compound may be administered to a subject by any convenient route of administration, whether systemically/ peripherally or at the site of desired action, including but not limited to, oral (e.g, by ingestion); topical (including e.g., transdermal, intranasal, ocular, buccal, and sublingual); pulmonary (e.g., by inhalation or insufflation therapy using, e.g., an aerosol, e.g., through mouth or nose); rectal; vaginal; parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot, for example, subcutaneously or intramuscularly.

The subject may be a eukaryote, an animal, a vertebrate animal, a mammal, a rodent (e.g., a guinea pig, a hamster, a rat, a mouse), murine (e.g., a mouse), canine (e.g., a dog), feline (e.g., a cat), equine (e.g., a horse), a primate, simian (e.g., a monkey or ape), a monkey (e.g., marmoset, baboon), an ape (e.g., gorilla, chimpanzee, orangutang, gibbon), or a human.

Formulations

While it is possible for the active compound to be administered alone, it is preferable to present it as a pharmaceutical composition (e.g., formulation) comprising at least one active compound, as defined above, together with one or more pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, preservatives, lubricants, or other materials well known to those skilled in the art and optionally other therapeutic or prophylactic agents.

Thus, the present invention further provides pharmaceutical compositions, as defined above, and methods of making a pharmaceutical composition comprising admixing at least one active compound, as defined above, together with one or more pharmaceutically acceptable carriers, excipients, buffers, adjuvants, stabilisers, or other materials, as described herein.

The term "pharmaceutically acceptable" as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g., human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

Suitable carriers, excipients, etc. can be found in standard pharmaceutical texts, for example, Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, Easton, Pa., 1990.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations may be in the form of liquids, solutions, suspensions, emulsions, elixirs, syrups, tablets, losenges, granules, powders, capsules, cachets, pills, ampoules, suppositories, pessaries, ointments, gels, pastes, creams, sprays, mists, foams, lotions, oils, boluses, electuaries, or aerosols.

Formulations suitable for oral administration (e.g., by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

A tablet may be made by conventional means, e.g., compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules, optionally mixed with one or more binders (e.g., povidone, gelatin, acacia, sorbitol, tragacanth, hydroxypropylmethyl

cellulose); fillers or diluents (e.g., lactose, microcrystalline cellulose, calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, silica); disintegrants (*e.g.*, sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose); surface-active or dispersing or wetting agents (e.g., sodium lauryl sulfate); and preservatives (e.g., methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, sorbic acid). Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active compound therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration (e.g., transdermal, intranasal, ocular, buccal, and sublingual) may be formulated as an ointment, cream, suspension, lotion, powder, solution, past, gel, spray, aerosol, or oil. Alternatively, a formulation may comprise a patch or a dressing such as a bandage or adhesive plaster impregnated with active compounds and optionally one or more excipients or diluents.

Formulations suitable for topical administration in the mouth include losenges comprising the active compound in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active compound in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active compound in a suitable liquid carrier.

Formulations suitable for topical administration to the eye also include eye drops wherein the active compound is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active compound.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of about 20 to about 500 microns which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid for administration as, for example, nasal spray, nasal drops, or by aerosol administration by nebuliser, include aqueous or oily solutions of the active compound.

Formulations suitable for administration by inhalation include those presented as an aerosol spray from a pressurised pack, with the use of a suitable propellant, such as dichlorodifluoromethane, trichlorofluoromethane, dichoro-tetrafluoroethane, carbon dioxide, or other suitable gases.

Formulations suitable for topical administration via the skin include ointments, creams, and emulsions. When formulated in an ointment, the active compound may optionally be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active compounds may be formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example, at least about 30% w/w of a polyhydric alcohol, i.e., an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol,

sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active compound through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogues.

When formulated as a topical emulsion, the oily phase may optionally comprise merely an emulsifier (otherwise known as an emulgent), or it may comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabiliser. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabiliser(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Suitable emulgents and emulsion stabilisers include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulphate. The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations may be very low. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP

may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active compound, such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration (e.g., by injection, including cutaneous, subcutaneous, intramuscular, intravenous and intradermal), include aqueous and non-aqueous isotonic, pyrogen-free, sterile injection solutions which may contain anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Examples of suitable isotonic vehicles for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the active compound in the solution is from about 1 ng/ml to about 10 μg/ml, for example from about 10 ng/ml to about 1 μg/ml. The formulations may be presented in unit-dose or

multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets. Formulations may be in the form of liposomes or other microparticulate systems which are designed to target the active compound to blood components or one or more organs.

Dosage

It will be appreciated that appropriate dosages of the active compounds, and compositions comprising the active compounds, can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects of the treatments of the present invention. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, and the age, sex, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, although generally the dosage will be to achieve local concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

Administration *in vivo* can be effected in one dose, continuously or intermittently (e.g., in divided doses at

appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

In general, a suitable dose of the active compound is in the range of about 100 μg to about 250 mg per kilogram body weight of the subject per day. Where the active compound is a salt, an ester, prodrug, or the like, the amount administered is calculated on the basis of the parent compound and so the actual weight to be used is increased proportionately.

EXAMPLES

The following are examples are provided solely to illustrate the present invention and are not intended to limit the scope of the invention, as described herein.

Synthesis Data

The compounds of which the structures are shown in Table 1, with a number greater than 100 were synthesised according to the synthesised routes above - the characterisation data follows. The compounds in Table 1 with a number less than 100 are available from Maybridge plc, Cornwall, UK.

Route 1 : Part 1 followed by Part 3 (A-B- = benzene ring)

101; Ar = 2,5-difluorophenyl

Yield, 58%; mpt. 277-280°C [DMF/EtOH]; d_H 7.40-7.60 (4H, m), 7.75-8.05 (2H, m), 8.30-8.45 (1H, m), 13.05 (1H, s); m/z $(M+H)^{+\cdot}$ 258 (100%).

103; Ar = 3-nitro-4-(4-chlorothiophenol)phenyl

Yield, 67%; mpt. 270-272°C [DMF/EtOH]; δ_H 7.05 (1H, d, J = 8 Hz), 7.60-8.05 (8H, m), 8.25-8.50 (2H, m), 13.00 (1H, s); m/z $(M-H)^{+\cdot}$ 410 (22%), 408 (10%).

104; Ar = 4-methoxyphenyl

Yield, 58%; mpt. 239-424°C [DMF/EtOH]; δ_H 3.80 (3H, s), 7.10 (2H, d, J = 7.5Hz), 7.55 (2H, d, J = 7.5Hz), 7.60-8.00 (3H, m), 8.25-8.45 (1H, m), 13.45 (1H, s); m/z $(M+H)^{+\cdot}$ 253 (100%).

116; Ar = 1-(2-methylbenzo[*b*]thiophene)

Yield, 70%; mpt. 288-290°C [DMF/EtOH]; δ_H 2.20 (3H, s), 7.40-7.70 (3H, m), 7.75-8.20 (4H, m), 8.25-8.50 (1H, m), 13.00 (1H, s); m/z $(M+H)^{+\cdot}$ 293 (28%).

119; Ar = 3-nitro-4-(2-aminothiophenol)phenyl

Yield, 39%; mpt. 223-225°C [DMF/EtOH]; δ_H 5.60 (2H, br s), 6.55-7.05 (3H, m), 7.15-7.50 (2H, m), 7.60-8.00 (4H, m), 8.20-8.50 (2H, m), 12.90 (1H, s); m/z $(M+H)^{+\cdot}$ 391 (26%).

120; Ar = 4-(*N*,*N*-dimethylamino)phenyl

Yield, 70%; mpt. 291-294°C [DMF/EtOH]; δ_H 3.00 (6H, s), 7.05 (1H, d, J = 5.70 Hz), 7.55 (1H, d, J = 5.25 Hz), 7.65-8.20 (6H, m), 8.25-8.45 (1H, m), 13.00 (1H, s); m/z $(M+H)^{+\cdot}$ 497 (100%).

128; Ar = 2-(5-methylthienyl)

Yield, 55%; mpt. 193-198°C [EtOH]; δ_H 2.15 (3H, s), 7.15 (1H, d, J = 6.30 Hz), 7.45-7.60 (1H, m), 7.75 (1H, d, J = 6.30 Hz), 7.90-8.10 (2H, m), 8.40-8.50 (1H, m), 13.00 (1H, br s).

137; Ar = 2-(5-bromothienyl)

Yield, 36%; mpt. 182-183°C [DMF/EtOH]; δ_H 7.35 (2H, d, J = 5.0 Hz), 7.50 (1H, d, J = 5.0 Hz), 7.80-8.20 (3H, m), 8.25-8.45 (1H, m), 13.00 (1H, s).

143; Ar = 3-(*N*-phenylsulfonyl)pyrrolo

Yield, 1%; mpt. 189-191°C [DMF/EtOH]; δ_H 6.75-6.80 (1H, m), 7.50-8.50 (11H, m), 13.00 (1H, s).

147; Ar = 2-furanyl

Yield, 36%; mpt. 153-155°C [DMF/EtOH]; δ_H 6.55-6.65 (1H, m), 6.85-6.95 (1H, m), 7.60-8.00 (3H, m), 8.25-8.45 (1H, m), 12.85 (1H, br s); m/z $(M+H)^{+\cdot}$ 213 (100%).

150; Ar = 4-bromophenyl

Yield, 79%; mpt. 212-214°C [DMF/EtOH]; δ_H 7.50-8.10 (7H, m), 8.25-8.45 (1H, m), 12.85 (1H, br s); m/z $(M+H)^{+\cdot}$ 301 (99%), 303 (95%).

152; Ar = 2-(5-methylthio)thienyl

Yield, 18%; mpt. 205-209°C [DMF/EtOH]; δ_H 2.60 (3H, s), 7.25 (1H, d, J = 5.0 Hz), 7.60 (1H, d, J = 5.0 Hz), 7.95-8.05 (2H, m), 8.10-8.20 (1H, m), 8.25-8.45 (1H, m), 12.85 (1H, br s); m/z $(M+H)^{+\cdot}$ 275 (100%).

153; Ar = 2-(5-*n*-butylthio)thienyl

Yield, 3%; mpt. 154-156°C [DMF/EtOH]; δ_H 1.00 (3H, t), 1.20-1.90 (4H, m), 2.85 (2H, t), 6.90 (1H, d, *J* = 4.60 Hz), 7.25 (1H, d, *J* = 4.60 Hz), 7.70-7.95 (2H, m), 8.10-8.20 (1H, m), 8.25-8.45 (1H, m), 13.00 (1H, br s); m/z $(M+H)^{+\cdot}$ 285 (100%).

161; Ar = 2-(5-phenylthio)thienyl

Yield, 26%; mpt. 161-163°C [DMF/EtOH]; δ_H 7.20-7.45 (5H, m), 7.45 (1H, d, *J* = 5.5 Hz), 7.60 (1H, d, *J* = 5.5 Hz), 7.95-8.05 (2H, m), 8.10-8.20 (1H, m), 8.25-8.45 (1H, m), 12.55 (1H, br s); m/z $(M+H)^{+\cdot}$ 337 (100%).

(-A-B- = naphthalene ring)

117; Ar = phenyl

Yield, 55%; mpt. 248-251°C [DMF/EtOH]; δ_H 7.00-7.10 (1H, m), 7.50-8.25 (8H, m), 8.35-8.50 (2H, m), 12.95 (1H, s); m/z $(M+H)^{+\cdot}$ 273 (27%).

(-A-B- = pyrazine ring)

146; Ar = phenyl

Yield, 33%; mpt. 178-180°C (dec.) [DMF/EtOH]; δ_H 7.25-7.60 (4H, m), 7.85-8.00 (1H, m), 8.60 (1H, s), 9.10 (1H, s); m/z $(M+H)^{+\cdot}$ 225 (100%).

Route 1: Part 1, followed by Part 2 and Part 3

(-A-B- = benzene ring)

108; Ar = 3-nitro-4-(*N*-morpholino)phenyl

Yield, 36%; mpt. 229-231°C [DMF/EtOH]; δ_H 2.95-3.15 (4H, m), 3.60-3.80 (4H, m), 7.45 (1H, d, *J* = 6.4 Hz), 7.60-8.10 (5H, m), 8.25-8.45 (1H, m), 12.85 (1H, s); m/z $(M+H)^{+\cdot}$ 353 (41%).

109; Ar = 3-nitro-4-(*N*-pyrollidino)phenyl

Yield, 10%; mpt. 243-245°C [DMF/EtOH]; δ_H 1.75-2.10 (4H, m), 3.05-3.45 (4H, m), 7.20 (1H, d, J = 6.4 Hz), 7.60-8.10 (5H, m), 8.25-8.45 (1H, m), 12.80 (1H, s); m/z $(M+H)^{+\cdot}$ 337 (100%).

110; Ar = 3-nitro-4-(*N*-[4-ethylpiperidine carboxylate]) phenyl

Yield, 46%; mpt. 192-193°C [DMF/EtOH]; δ_H 1.20 (3H, t), 1.50-2.00 (4H, m), 2.50-2.75 (1H, m), 2.80-3.50 (4H, m), 4.10 (2H, q), 7.50 (1H, d, J = 5.7 Hz), 7.65-8.00 (5H, m), 8.25-8.45 (1H, m), 12.75 (1H, s); m/z $(M+H)^{+\cdot}$ 423 (100%).

111; Ar = 3-nitro-4-(*N*-thiomorpholino)phenyl

Yield, 38%; mpt. 217-219°C [DMF/EtOH]; δ_H 2.65-2.75 (4H, m), 3.20-3.50 (4H, m), 7.50 (1H, d, J = 5.6 Hz), 7.60-8.10 (5H, m), 8.30-8.50 (1H, m), 12.90 (1H, s); m/z $(M-H)^{+\cdot}$ 367 (100%).

112; Ar = 3-nitro-4-(*N*, *N*-di-*n*-butylamino)phenyl

Yield, 25%; mpt. 156-160°C [DMF/EtOH]; δ_H 0.80 (6H, t), 1.00-1.65 (8H, m), 3.20 (4H, t), 7.45 (1H, d, J = 6.0 Hz), 7.60-8.00 (5H, m), 8.35-8.50 (1H, m), 12.80 (1H, s); m/z $(M-H)^{+\cdot}$ 393 (36%).

113; Ar = 3-nitro-4-(*N*-[4-amidopiperidino])phenyl

Yield, 68%; mpt. 227-230°C [DMF/EtOH]; δ_H 1.60-2.00 (4H, m), 2.10-2.45 (1H, m), 2.65-3.10 (2H, m), 3.15-3.45 (2H, m), 7.05 (1H, d, J = 30 Hz), 7.50 (1H, d, J = 5.4 Hz), 7.60-8.10 (5H, m), 8.25-8.45 (1H, m), 13.00 (1H, s); m/z $(M-H)^{+\cdot}$ 392 (100%).

133; Ar = 3-nitro-4-(*N*-homopiperidino)phenyl

Yield, 46%; mpt. 181-183°C [DMF/EtOH]; δ_H 1.60-200 (2H, m), 2.70-3.00 (2H, m), 3.05-3.75 (6H, m), 7.40 (1H, d, *J* = 8.50 Hz), 7.60-8.00 (5H, m), 8.25-8.50 (1H, m) m/z $(M-H)^{+\cdot}$ 364 (100%).

156; Ar = 3-nitro-4-pyrrolophenyl

Yield, 22%; mpt. 244-247°C [DMF/EtOH]; δ_H 6.25-6.35 (2H, m), 7.00-7.05 (2H, m), 7.60-8.00 (5H, m), 8.25-8.45 (2H, m); m/z $(M-H)^{+\cdot}$ 331 (100%).

157; Ar = 4-hydroxy-3-nitrophenyl

Yield, 13%; mpt. >300°C [DMF]; δ_H 7.10 (1H, d, *J* = 7.7 Hz), 7.50-8.00 (5H, m), 8.20-8.40 (1H, m); m/z $(M-H)^{+\cdot}$ 282 (100%).

Route 2 (-A-B- = benzene ring)

126; Ar = 4-chlorophenyl

Yield, 31%; mpt. 218-220°C; δ_H 4.30 (2H, s), 7.30 (4H, s), 7.75-8.00 (3H, s), 8.25-8.45 (1H, m), 12.40 (1H, br s); m/z $(M+H)^{+\cdot}$ 271 (100%), 273 (35%).

129; Ar = 4-bromophenyl

Yield, 59%; mpt. 232-235°C; δ_H 4.40 (2H, s), 7.30 (2H, d, *J* = 8.7 Hz), 7.45 (2H, d, *J* = 8.7 Hz), 7.60-7.95 (3H, m), 8.25-8.45 (1H, m), 12.40 (1H, br s); m/z $(M+H)^{+\cdot}$ 314 (100%), 316 (95%).

131; Ar = 1-naphthyl

Yield, 58%; mpt. 228-231°C; δ_H 4.80 (2H, s), 7.25-8.50 (11H, m), 12.50 (1H, br s); m/z $(M+H)^{+\cdot}$ 287 (100%).

132; Ar = 4-fluorophenyl

Yield, 54%; mpt. 194-197°C; δ_H 4.30 (2H, s), 7.10 (2H, d, *J* = 8.5 Hz), 7.25 (2H, d, *J* = 8.5 Hz), 7.25-7.50 (1H, m), 7.75-8.00 (2H, m), 8.25-8.45 (1H, m), 12.55 (1H, br s); m/z $(M+H)^{+\cdot}$ 255 (100%).

138; Ar = 4-methoxyphenyl

Yield, 66%; mpt. 194-196°C; δ_H 3.70 (3H, s), 4.50 (2H, s), 6.85 (2H, d, *J* = 8.5 Hz), 7.30 (2H, d, *J* = 8.5 Hz), 7.70-8.00 (3H, m), 8.25-8.45 (1H, m), 12.50 (1H, br s); m/z $(M+H)^{+\cdot}$ 267 (100%).

139; Ar = 4-methylphenyl

Yield, 80%; mpt. 205-207°C; δ_H 2.15 (3H, s), 4.25 (2H, s), 7.00-7.30 (4H, m), 7.60-7.95 (3H, m,), 8.25-8.45 (1H, m), 12.60 (1H, br s); m/z $(M+H)^{+\cdot}$ 251 (100%).

141; Ar = 2-fluorophenyl

Yield, 85%; mpt. 235-238°C; δ_H 4.40 (2H, s), 7.10-7.45 (4H, m), 7.70-8.05 (3H, m), 8.25-8.45 (1H, m), 12.40 (1H, br s); m/z $(M+H)^{+\cdot}$ 255 (100%).

142; Ar = 2-methoxyphenyl

Yield, 74%; mpt. 158-160°C; δ_H 3.70 (3H, s), 4.25 (2H, s), 6.70-6.95 (3H, m), 7.10-7.35 (1H, m), 7.60-7.95 (3H, m), 8.45-8.55 (1H, m), 11.15 (1H, br s); m/z $(M+H)^{+\cdot}$ 281 (100%).

145; Ar = phenyl

Yield, 85%; mpt. 201-204°C; δ_H 4.45 (2H, s), 7.20-7.45 (5H, m), 7.75-8.00 (3H, m), 8.25-8.45 (1H, m), 12.40 (1H, br s); m/z $(M+H)^{+\cdot}$ 237 (100%).

151; Ar = 4-iodophenyl

Yield, 86%; mpt. 233-236°C; δ_H 4.20 (2H, s), 7.15 (2H, d, *J* = 8.2 Hz), 7.60 (2H, d, *J* = 8.2 Hz), 7.75-7.95 (3H, m), 8.25-8.45 (1H, m), 12.15 (1H, br s); m/z $(M+H)^{+\cdot}$ 362 (100%).

159; Ar = 4-aminophenyl

Yield, 28%; mpt. 233-236°C; δ_H 4.15 (2H, s), 4.85 (2H, s), 6.50 (2H, d, *J* = 7.1 Hz), 7.00 (2H, d, *J* = 7.1 Hz), 7.75-7.95 (3H, m), 8.25-8.45 (1H, m), 12.50 (1H, br s); m/z $(M+H)^{+\cdot}$ 252 (100%).

160; Ar = 3-aminophenyl

Yield, 95%; mpt. 178-180°C; δ_H 4.15 (2H, s), 5.00 (2H, br s), 6.35-6.55 (3H, m), 6.80-7.05 (1H, m), 7.75-7.90 (3H, m), 8.25-8.40 (1H, m); m/z $(M+H)^{+\cdot}$ 252 (100%).

163; Ar = 2-methylphenyl

Yield, 72%; mpt. 201-204°C; δ_H 2.15 (3H, s), 4.10 (2H, s), 6.95-7.25 (4H, m), 7.80-7.95 (3H, m), 8.25-8.45 (1H, m), 12.25 (1H, br s).

177; Ar = 4-pyridyl

Yield, 40%; mpt. 214-216°C; δ_H 4.25 (2H, s), 7.45 (2H, d, *J* = 5.7 Hz), 7.75-7.95 (3H, m), 8.25-8.45 (1H, m), 8.55 (2H, d, *J* = 5.7 Hz), 12.00 (1H, br s); m/z $(M+H)^{+\cdot}$ 238 (100%).

178; Ar = 3-pyridyl

Yield, 62%; mpt. 196-199°C; δ_H 4.30 (2H, s), 7.25-7.45 (1H, m), 7.60-7.95 (4H, m), 8.25-8.45 (2H, m), 8.55 (1H, s), 12.15 (1H, br s); m/z $(M+H)^{+\cdot}$ 238 (100%).

180; Ar = 3,4-methylenedioxyphenyl

Yield, 59%; mpt. 225-228°C; δ_H 4.25 (2H, s), 6.00 (2H, s), 6.85-7.00 (3H, m), 7.70-7.95 (3H, m), 8.25-8.45 (1H, m), 12.25 (1H, br s); m/z $(M+H)^{+\cdot}$ 281 (100%).

Route 3 (-A-B- = benzene ring)

123; Ar = *N*-imidazole

Yield, 25%; mpt. 280-283°C [EtOH]; δ_H 7.90-8.05 (6H, m), 8.25-8.45 (1H, m), 12.85 (1H, br s); m/z $(M+H)^{+\cdot}$ 213 (22%).

130; Ar = *N*-1,2,4-triazole

Yield, 26%; mpt. 285-287°C [EtOH]; δ_H 7.80-8.20 (3H, m), 8.30-8.60 (2H, m), 9.10-9.25 (1H, br s).

Further derivatisation steps (-A-B- = benzene ring)

(a)

121; Ar = 3-amino-4-(*N*-piperidine)phenyl

Yield, 49%; mpt. 241-244°C [DMF/EtOH]; δ_H 1.50-1.90 (6H, m), 2.80-3.00 (4H, m), 4.95 (2H, br s), 6.65-7.15 (3H, m), 7.80-7.95 (3H, m), 8.25-8.45 (1H, m), 12.85 (1H, br s); m/z $(M+H)^{+\cdot}$ 320 (100%).

144; Ar = 3-amino-4-(*N*-thiomorpholine)phenyl

Yield, 30%; mpt. >320°C [DMF/EtOH]; δ_H 2.75-3.00 (4H, m), 3.00-3.25 (4H, m), 5.00 (2H, br s), 6.75-7.15 (2H, m), 7.60-8.00 (4H, m), 8.25-8.50 (1H, m), 12.85 (1H, br s); m/z $(M+H)^{+\cdot}$ 335 (100%).

148; Ar = 3-amino-4-(*N*-morpholino)phenyl

Yield, 27%; mpt. 265-268°C [DMF/EtOH]; δ_H 2.75-3.00 (4H, m), 3.00-3.25 (4H, m), 5.00 (2H, br s), 6.70-7.10 (2H, m), 7.60-8.00 (4H, m), 8.25-8.50 (1H, m), 12.65 (1H, br s); m/z $(M+H)^{+\cdot}$ 322 (100%).

162; Ar = 3-amino-4-(*N*-pyrrolidino)phenyl

Yield, 17%; mpt. 237-240°C [DMF/EtOH]; δ_H 1.90-2.00 (4H, m), 2.95-3.10 (4H, m), 4.70 (2H, br s), 6.60-7.00 (3H, m), 7.70-8.00 (3H, m), 8.25-8.50 (1H, m), 12.50 (1H, br s); m/z $(M+H)^{+\cdot}$ 307 (100%).

168; Ar = 3-amino-4-hydroxyphenyl

Yield, 5%; mpt. 244-247°C [DMF/EtOH]; δ_H 5.20 (2H, br s), 6.50-6.80 (3H, m), 7.75-7.95 (3H, m), 8.25-8.40 (1H, m), 9.40 (1H, br s), 12.50 (1H, s); m/z $(M+H)^{+\cdot}$ 254

176; Ar = 3-amino-4-(*N*-pyrrolo)phenyl

Yield, 7%; mpt. 230-239°C [DMF/EtOH]; Impure mixture. By LC approx. 25% product, 25% starting material, and 50% **109**; m/z $(M+H)^{+\cdot}$ 335 (100%).

(b)

155; $NR_{N1}R_{N2}$ = piperidine; R′ = acetyl

Yield, 36%; mpt. 277-280°C [EtOH]; δ_H 1.50-1.90 (6H, m), 2.05 (3H, s), 2.80-3.00 (4H, m), 6.65-7.15 (3H, m), 7.80-7.95 (3H, m), 8.25-8.45 (1H, m), 9.00 (1H, s), 12.85 (1H, br s); m/z $(M+H)^{+\cdot}$ 363 (100%).

158; $NR_{N1}R_{N2}$ = morpholine; R′ = acetyl

Yield, 32%; mpt. 272-275°C [EtOH]; δ_H 2.05 (3H, s), 2.80-3.00 (4H, m), 3.90-4.00 (4H, m), 7.15 (2H, s), 7.80-8.20

(5H, m), 9.00 (1H, s), 12.85 (1H, s); m/z $(M+H)^{+\cdot}$ 365 (100%).

173; $NR_{N1}R_{N2}$ = pyrrolidine; R' = 4-fluorobenzoyl
Yield, 86%; mpt. 263-267°C [EtOH]; δ_H 1.60-1.95 (4H, m), 3.10-3.40 (4H, m), 6.75-7.50 (5H, m), 7.55-8.10 (5H, m), 8.25-8.45 (1H, m), 9.85 (1H, s), 12.55 (1H, br s); m/z $(M+H)^{+\cdot}$ 429 (100%).

174; $NR_{N1}R_{N2}$ = pyrrolidine; R' = 2-thienylcarboyl
Yield, 90%; mpt. 219-223°C (dec.) [EtOH]; δ_H 1.60-1.95 (4H, m), 3.10-3.40 (4H, m), 6.90-7.00 (1H, m), 7.15-7.50 (3H, m), 7.60-8.00 (6H, m), 8.25-8.45 (1H, m), 9.90 (1H, s), 12.65 (1H, br s); m/z $(M+H)^{+\cdot}$ 417 (100%).

175; $NR_{N1}R_{N2}$ = pyrrolidine; R' = benzoyl
Yield, 58%; mpt. 252-256°C [EtOH]; δ_H 1.60-1.95 (4H, m), 3.10-3.40 (4H, m), 6.95 (1H, d), 7.15-7.50 (4H, m), 7.70-8.00 (6H, m), 8.25-8.45 (1H, m), 10.00 (1H, s), 12.65 (1H, s); m/z $(M+H)^{+\cdot}$ 411 (100%).

181; $NR_{N1}R_{N2}$ = piperidine; R' = benzoyl
Yield, 83%; mpt. 258-262°C [EtOH]; δ_H 1.60 (6H, br s), 3.00 (4H, br s), 7.45 (1H, s), 7.50-7.60 (3H, m), 7.75-8.00 (6H, m), 8.25-8.45 (2H, m), 9.75 (1H, s), 12.65 (1H, br s).

182; $NR_{N1}R_{N2}$ = piperidine; R' = 4-fluorobenzoyl
Yield, 86%; mpt. 285-289°C [EtOH]; δ_H 1.60 (6H, br s), 3.00 (4H, br s), 7.25-7.50 (3H, m), 7.75-8.15 (6H, m), 8.25 (2H, s), 9.75 (1H, s), 12.65 (1H, br s).

184; $NR_{N1}R_{N2}$ = piperidine; R' = 4-fluorophenylsulfonyl

Yield, 60%; mpt. 243-247°C [EtOH]; δ_H 1.60 (6H, br s), 3.00 (4H, br s), 7.25-7.60 (5H, m), 7.75-8.00 (5H, m), 8.25-8.45 (1H, m), 9.00 (1H, br s), 12.65 (1H, br s).

(c)

125; R_C = 4-hydroxy phenyl

Yield, 88%; mpt. 304-306°C; δ_H 3.45 (2H, s), 6.95 (2H, d, *J* = 7.4 Hz), 7.40 (2H, d, *J* = 7.4 Hz), 7.60-8.00 (3H, m), 8.25-8.45 (1H, m), 9.80 (1H, s), 12.55 (1H, br s); m/z $(M+H)^{+\cdot}$ 239 (100%).

(d)

135; R_C = 3-nitro-4-(*N*-[4-piperidinecarboxylate])phenyl

Yield, 77%; mpt. 276-278°C (dec.) [DMF/EtOH]; δ_H 1.50-2.00 (4H, m), 2.50-2.75 (1H, m), 2.80-3.50 (4H, m), 7.50 (1H, d, *J* = 5.7 Hz), 7.65-8.00 (5H, m), 8.25-8.45 (1H, m), 12.75 (1H, s); m/z $(M+H)^{+\cdot}$ 395 (7%).

(e)

107; R_N = 2-thienylsulfonyl (where R_N-X = 2-thienylsulfonyl chloride)

Yield, 20%; mpt. 195-199°C [DMF/EtOH]; δ_H 7.25-7.40 (1H, m), 7.60 (5H, s), 7.90-8.45 (6H, m).

127; R_N = methanesulfonyl (where R_N-X = methanesulfonyl chloride)

Yield, 50%; mpt. 201-203°C [DMF/EtOH]; δ_H 3.80 (3H, s), 7.60 (5H, s), 7.60-7.85 (1H, m), 7.95-7.15 (2H, m), 8.25-8.45 (1H, m).

140; R_N = methyl (where R_N-X = methyl iodide)

Yield, 96%; mpt. 188-190°C [DMF/EtOH]; δ_H 3.80 (3H, s), 7.60 (5H, s), 7.60-7.65 (1H, m), 7.95-7.15 (2H, m), 8.25-8.45 (1H, m).

(f)

164; 4-methoxy

Yield, 99%; mpt. 231-234°C; δ_H 4.15 (2H, s), 6.60 (2H, d, *J* = 8.0 Hz), 7.10 (2H, d, *J* = 8.0 Hz), 7.80-7.95 (3H, m), 8.25-8.45 (1H, m), 12.50 (1H, s); m/z $(M+H)^{+\cdot}$ 253 (100%).

165; 3-methoxy

Yield, 68%; mpt. 198-201°C; δ_H 4.15 (2H, s), 6.50-6.80 (3H, m,), 6.95-7.10 (1H, m), 7.80-7.95 (3H, m), 8.25-8.45 (1H, m), 12.50 (1H, s); m/z $(M+H)^{+\cdot}$ 253 (100%).

(g)

166; R" = 4-NHC(=O)CH_3

Yield, 57%; mpt. 267-271°C; δ_H 2.00 (3H, s), 4.25 (2H, s), 7.25 (2H, d, *J* = 7.7 Hz), 7.55 (2H, d, *J* = 7.7 Hz), 7.75-7.95 (3H, m), 8.25-8.45 (1H, m), 9.80 (1H, s), 12.50 (1H, br s); m/z $(M+H)^{+\cdot}$ 294 (100%).

167; R" = 4-NHC(=O)Ph

Yield, 87%; mpt. 293-296°C; δ_H 4.25 (2H, s), 7.20-8.00 (12H, m), 8.25-8.45 (1H, m), 10.15 (1H, s), 12.50 (1H, s); m/z $(M+H)^{+\cdot}$ 356 (100%).

169; R″ = 3-NHC(=O)-2-thienyl

Yield, 72%; mpt. 232-235°C; δ_H 4.25 (2H, s), 7.00-7.45 (3H, m), 7.55-7.65 (2H, m), 7.75-7.95 (5H, m), 8.25-8.45 (1H, m), 10.10 (1H, s), 12.50 (1H, br s); m/z $(M+H)^{+\cdot}$ 362 (100%).

170; R″ = 3-NHC(=O)-4-fluorophenyl

Yield, 68%; mpt. 257-261°C; δ_H 4.25 (2H, s), 7.00-7.50 (4H, m), 7.55-8.25 (8H, m), 10.15 (1H, s), 12.50 (1H, br s); m/z $(M+H)^{+\cdot}$ 374 (100%).

171; R″ = 3-NHC(=O)Ph

Yield, 78%; mpt. 261-264°C; δ_H 4.25 (2H, s), 7.05-7.95 (12H, m), 10.05 (1H, s), 12.50 (1H, s); m/z $(M+H)^{+\cdot}$ 356 (100%).

172; R″ = 3-NHC(=O)CH_3

Yield, 55%; mpt. 270-272°C; δ_H 2.00 (3H, s), 4.25 (2H, s), 7.00-7.50 (4H, m), 7.75 (3H, s), 8.25-8.45 (1H, m), 9.80 (1H, s), 12.50 (1H, br s); m/z $(M+H)^{+\cdot}$ 294 (100%).

(h)

179

Yield, 45%; mpt. 161-164°C; δ_H 2.15 (3H, s), 4.25 (2H, s), 6.90-7.35 (4H, m), 7.75-7.95 (3H, s), 8.25-8.45 (1H, m), 12.50 (1H, br s); m/z $(M+H)^{+\cdot}$ 295 (100%).

(i)

149

Yield, 90%; mpt. 243-245°C; δ_H 6.50-6.55 (1H, m), 6.90-6.95 (1H, m), 7.20-7.40 (1H, m), 7.85-8.00 (2H, m), 8.25-8.45 (2H, m), 11.10 (1H, br s), 12.50 (1H, s); m/z $(M+H)^{+\cdot}$ 212 (100%).

Biological Testing

In order to assess the inhibitory action of the compounds, the following assay was used to determine IC_{50} values.

Mammalian PARP, isolated from Hela cell nuclear extract, was incubated with Z-buffer (25mM Hepes (Sigma); 12.5 mM $MgCl_2$ (Sigma); 50mM KCl (Sigma); 1 mM DTT (Sigma); 10% Glycerol (Sigma) 0.001% NP-40 (Sigma); pH 7.4) in 96 well FlashPlates (TRADE MARK) (NEN, UK) and varying concentrations of said inhibitors added. All compounds were diluted in DMSO and gave final assay concentrations of between 10 and 0.01 μM, with the DMSO being at a final concentration of 1% per well. The total assay volume per well was 40 μl.

After 10 minutes incubation at 30°C the reactions were initiated by the addition of a 10 μl reaction mixture, containing NAD (5μM), ^{3}H-NAD and 30mer double stranded DNA-oligos. Designated positive and negative reaction wells were done in combination with compound wells (unknowns) in order to calculate % enzyme activities. The plates were then shaken for 2 minutes and incubated at 30°C for 45 minutes.

Following the incubation, the reactions were quenched by the addition of 50 μl 30% acetic acid to each well. The plates were then shaken for 1 hour at room temperature.

The plates were transferred to a TopCount NXT (TRADE MARK) (Packard, UK) for scintillation counting. Values recorded are counts per minute (cpm) following a 30 second counting of each well.

The % enzyme activity for each compound is then calculated using the following equation:

$$\% \text{ Inhibition} = 100 - \left(100x\frac{(\text{cpm of unknowns} - \text{mean negative cpm})}{(\text{mean positive cpm} - \text{mean neagative cpm})}\right)$$

The results are detailed below in Table 1 as IC_{50} values (the concentration at which 50% of the enzyme activity is inhibited), which are determined over a range of different concentrations, normally from 10 μM down to 0.01 μM. Such IC_{50} values are used as comparative values to identify increased compound potencies.

For comparison, the IC_{50} of 100 (1(2H)-phthalazinone was determined using the above test to be 7.2 μM.

The Dose Enhancing Factor (DEF) is a ratio of the enhancement of cell growth inhibition elicited by the test compound in the presence of bleomycin compared to bleomycin alone. The test compounds were used at a fixed concentration of 25 μM. Bleomycin was used at a concentration of 0.5 μg/ml. The DEF was calculated from the formula:

$$\frac{\text{Growth}_{TC}}{\text{Growth}_{control}} x \frac{\text{Growth}_{bleo}}{\text{Growth}_{(bleo+TC)}}$$

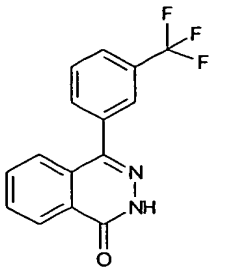
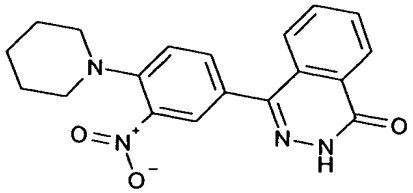
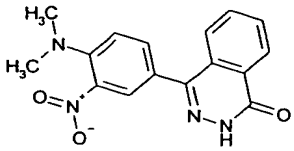
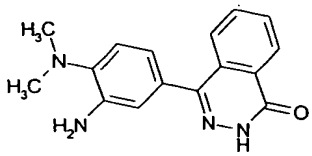
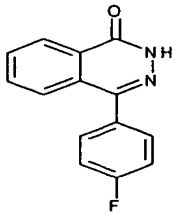
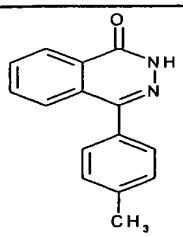
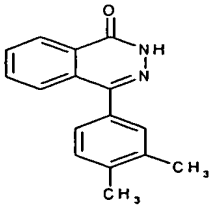
where Growth_{TC} is cell growth in presence of the test compound;
$\text{Growth}_{Control}$ is cell growth of control cells;
Growth_{bleo} is cell growth in presence of bleomycin; and
$\text{Growth}_{(bleo+TC)}$ is cell growth in presence of bleomycin and the test compound.

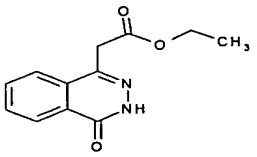
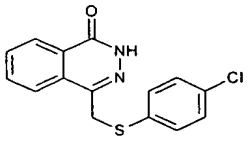
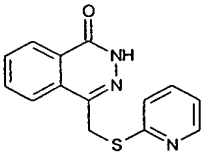
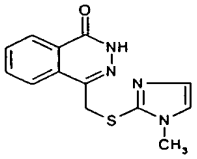
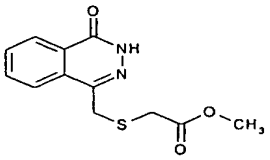
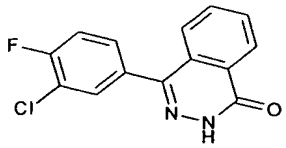
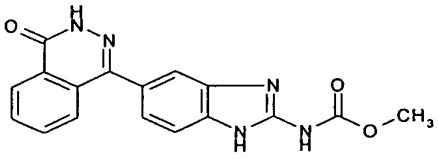
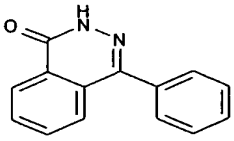
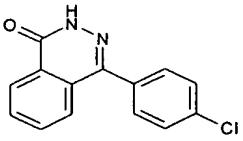
Cell growth was assessed using the sulforhodamine B (SRB) assay (Skehan, P., *et al.*, 1990, *J. Natl. Cancer Inst.*, **82**, 1107-1112). 2,000 HeLa cells were seeded into each well of a flat-bottomed 96-well microtiter plate in a volume of 100 µl and incubated for 6 hours at 37°C. Cells were either replaced with media alone or with media containing the test compound at a final concentration of 25 µM. Cells were allowed to grow for a further 1 hour before the addition of bleomycin to either untreated cells or test compound treated cells. Cells untreated with either bleomycin or test compound were used as a control. Cells treated with test compound alone were used to assess the growth inhibition by the test compound.

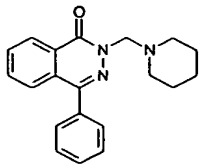
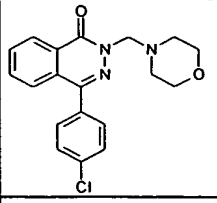
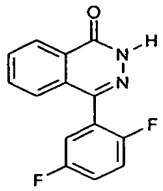
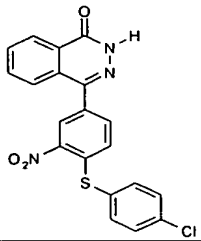
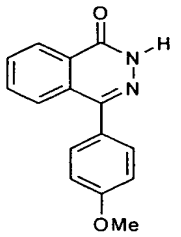
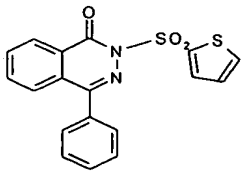
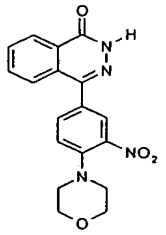
Cells were left for a further 16 hours before replacing the media and allowing the cells to grow for a further 72 hours at 37°C. The media was then removed and the cells fixed with 100µl of ice cold 10% (w/v) trichloroacetic acid. The plates were incubated at 4°C for 20 minutes and then washed four times with water. Each well of cells was then stained with 100µl of 0.4% (w/v) SRB in 1% acetic acid for 20 minutes before washing four times with 1% acetic acid. Plates were then dried for 2 hours at room temperature. The dye from the stained cells was solubilized by the addition of 100µl of 10mM Tris Base into each well. Plates were gently shaken and left at room temperature for 30 minutes before measuring the optical density at 564nM on a Microquant microtiter plate reader.

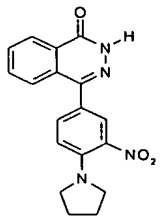
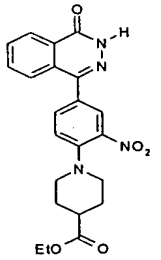
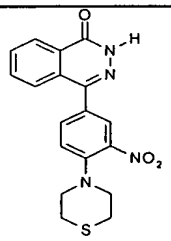
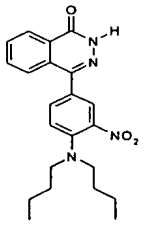
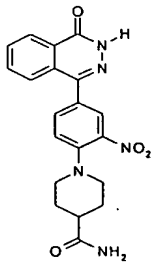
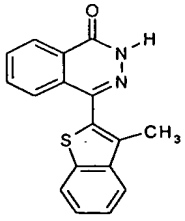
The results are shown in Table 1 below.

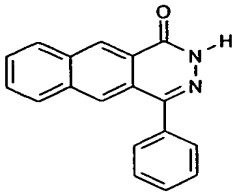
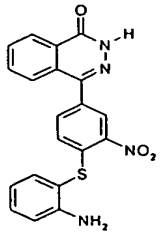
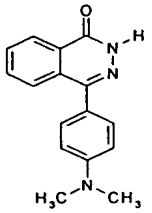
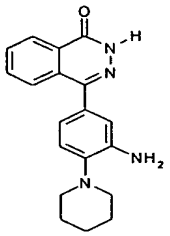
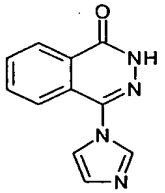
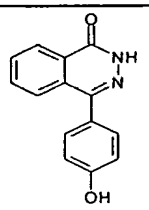
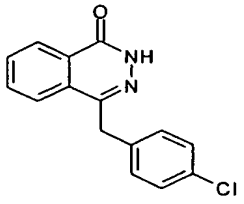
Table 1

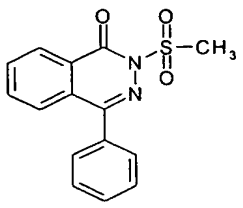
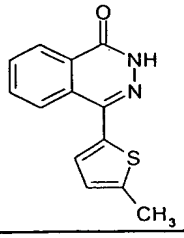
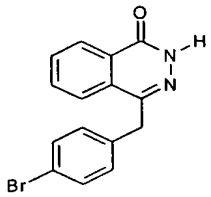
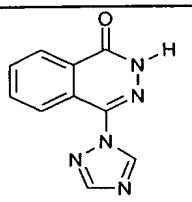
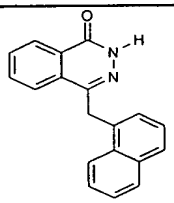
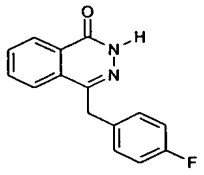
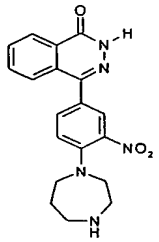
Compound No.	Structure	IC50 (microM)	DEF
82		3.4	1.2
83		1.7	1.1
84		2.6	1.5
85		0.9	1.5
86		2.2	1.2
87		2.2	1.2
88		1.1	1.2

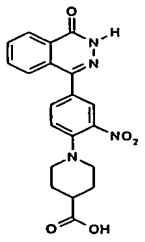
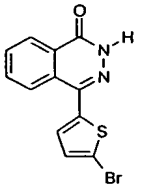
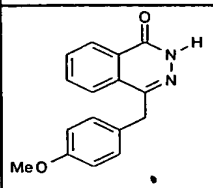
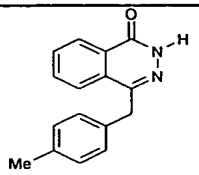
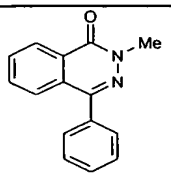
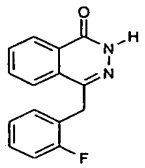
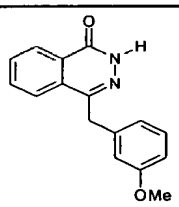
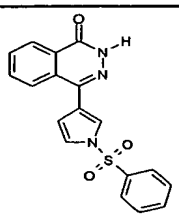
89		1.7	1.4
90		0.9	1.8
91		3.3	1.4
92		1.3	1.5
93		2.1	1.3
94		2.7	1.3
95		1.6	1.8
96		1	1.3
97		5.3	1.2

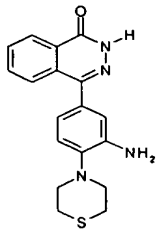
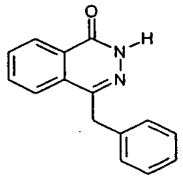
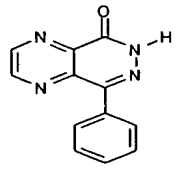
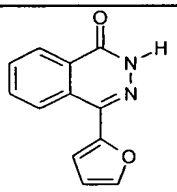
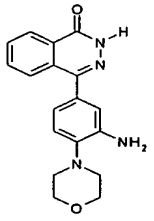
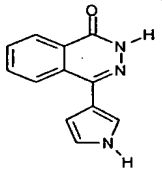
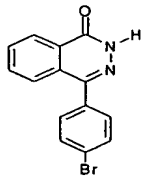
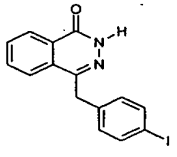
98		6.1	-
99		4.7	-
101		5.8	-
103		4	-
104		1.1	1.4
107		-	-
108		1	-

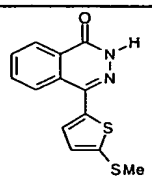
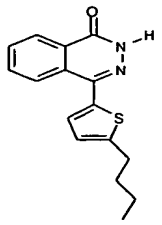
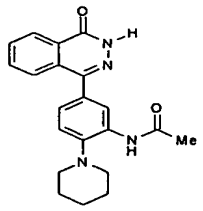
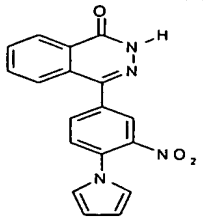
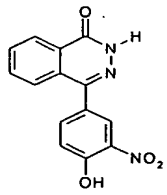
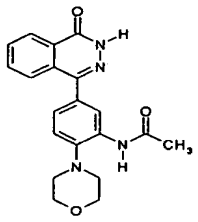
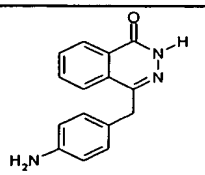
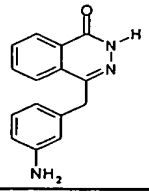
109		0.5	1.6
110		1.9	-
111		0.6	1.4
112		-	-
113		1.6	-
116		-	-

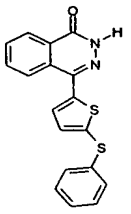
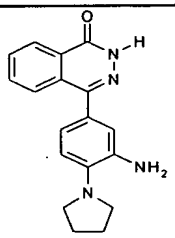
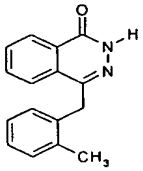
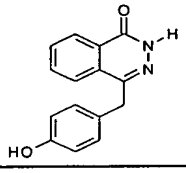
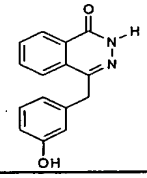
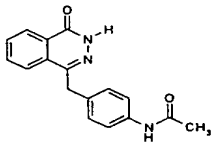
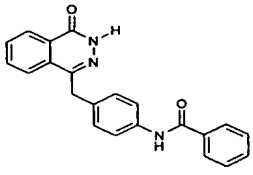
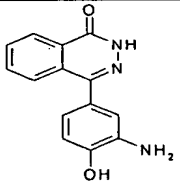
117		6.7	-
119		3.6	-
120		3.1	-
121		0.09	1.6
123		2.2	1
125		0.8	1.5
126		1.8	1.9

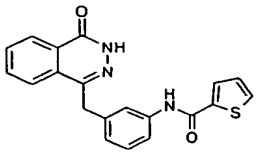
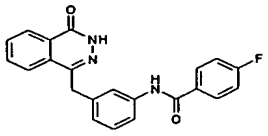
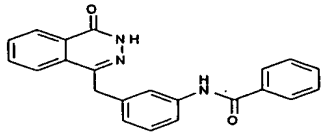
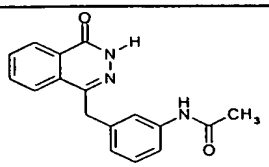
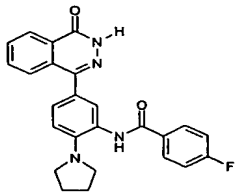
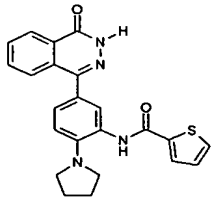
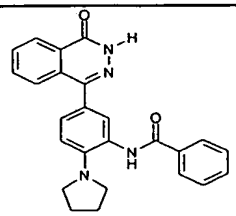
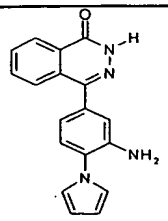
127		-	-
128		1.9	1.6
129		1.6	1.3
130		-	-
131		4.4	1.3
132		0.7	2.3
133		0.5	1.6

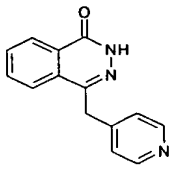
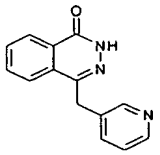
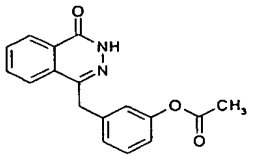
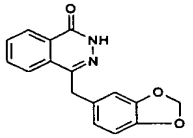
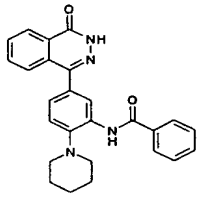
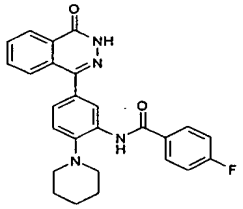
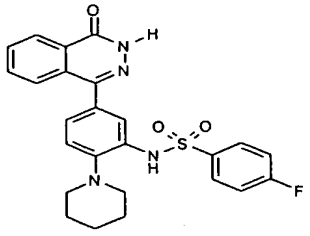
135		2.9	1.4
137		0.6	1.4
138		1.2	1.3
139		1.7	1.4
140		-	-
141		1.4	1.6
142		0.7	1.6
143		0.5	1.3

144		0.6	1.9
145		0.8	1.8
146		-	-
147		0.6	1.1
148		0.2	1.7
149		1	1.7
150		0.7	1
151		1.8	1.9

152		0.5	1.8
153		0.5	1.6
155		0.7	1.4
156		0.6	1.7
157		7.2	-
158		1.4	1.6
159		3.5	-
160		1.3	1.5

161		0.5	1.3
162		0.2	1.7
163		1.8	1.3
164		1.8	1.3
165		0.3	1.3
166		4.1	1.4
167		1.6	2.2
168		0.6	2.2

169		0.6	1.5
170		0.4	2.6
171		0.6	1.8
172		0.09	1.4
173		0.6	1.8
174		0.5	1.2
175		0.5	1.6
176		0.5	1.4

177		0.8	1.2
178		0.21	1.9
179		0.04	1.4
180		1.3	1.2
181		1.2	2
182		8	2.7
184		0.8	3